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Biosynthesis of Psilocybin

Part II.* Incorporation of Labelled Tryptamine Derivatives

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The biosynthesis of psilocybin has been investigated by feeding labelled precursors to *Psilocybe cubensis*. The following specifically labelled compounds were synthesized: psilocin-^{3}H, 4-hydroxytryptamine-^{14}C, *N*,*N*-dimethyltryptamine-^{14}C and -^{14}C-^{3}H, *N*-methyltryptamine-^{14}C-^{3}H and DL-tryptophan-^{3}H. A number of other indoles were labelled by acid catalyzed exchange in tritiated water. The experimental data suggest a sequence: tryptophan ⟶ tryptamine ⟶ *N*-methyltryptamine ⟶ *N*,*N*-dimethyltryptamine ⟶ psilocin ⟶ psilocybin. The fungus can also by an alternative route convert 4-hydroxytryptamine to psilocybin. Large differences in the rate of absorption of different closely related precursors has been observed.

Mushrooms containing the hallucinogenic compounds psilocybin (XVI) and psilocin (IV) have long been used as intoxicants by the natives of Mexico. The compounds are closely related 4-hydroxylated indole derivatives [1] and occur mainly in members of the genus *Psilocybe*. The production of psilocybin in *Psilocybe* species in submerged culture was sucessfully accomplished by Catalfomo and Tyler Jr.[2] and recently also by Leung *et al.*[3]

Brack *et al.*[4] found that psilocybin was biosynthesized from tryptophan in still cultures of *P. semperviva*. In our previous study [5] we found that submerged cultures of *P. cubensis* effectively incorporated also tryptamine into psilocybin.

The present study was undertaken to clarify the sequence of events which leads from tryptophan to psilocybin. This sequence will involve the following modifications of tryptophan in a definite or alternative order: decarboxylation, *N*-methylation, 4-hydroxylation, and *O*-phosphorylation (Fig. 3).

To elucidate this pathway we have used two approaches. In the present study we have investigated the incorporation into psilocybin of labelled

* Part I. Ref. 5.

hypothetical intermediates. The subsequent paper [6] is concerned with a study of the conversion of tryptophan to psilocybin and the isolation of "apparent intermediates" in this process.

EXPERIMENTAL

Melting points were determined with an electrically heated metal block using calibrated Anschütz thermometers. Infrared spectra of synthesized labelled compounds were recorded with a Perkin-Elmer 237 spectrophotometer and if possible compared with references.

Sodium cyanide-^{14}C, L-tryptophan-G-^{3}H, DL-tryptophan-1'-^{14}C, and methyl iodide-^{14}C were purchased from the Radiochemical Center, Amersham, and $LiAlH_4$—^{3}H and tryptamine-2'-^{14}C bisuccinate from New England Nuclear Corporation, Boston. The purity of the commercially available and synthesized labelled compounds was checked by thin-layer and paper chromatography followed by scanning in a chromatogram scanner.[7] When necessary, labelled intermediates and final products were purified by preparative paper [5] or column [6] chromatography. Reference materials were obtained from Calbiochem, Los Angeles; Sandoz AG, Basel; and Koch-Light, Colnbrook.

Psilocin-2'-^{3}H (IV). Fig. 1.

4-Benzyloxy-3-(N,N-dimethylcarbamoylmethyl)indole (II) was prepared from 4-benzyloxyindolyl-(3)-acetic acid (I) *via* the acid chloride.[8] Yield 64 %; m.p. 180—181°. Reported [8] m.p. 175—178°.

4-Benzyloxy-3-(2-dimethylaminoethyl)indole-2'-^{3}H (III). 0.7 g II (2. 28 mM) in 20 ml dry tetrahydrofuran was slowly added to 0.35 g (9.24 mmoles) tritiated $LiAlH_4$ (2.5 mC) in 20 ml tetrahydrofuran. After one hour at 40°, 5 ml water was added and the mixture stirred for 20 min. 15 ml of 20 % NaOH was added and the turbid solution was extracted with ether. After drying over Na_2SO_4 and evaporation of solvent, the residue crystallized. Yield 0.55 g (84 %); m.p. 120—121°. Reported [8] m.p. 120—121°.

Psilocin-2'-^{3}H (IV) = 3-(2-methylaminoethyl)-4-hydroxyindole-^{3}H. 0.5 g III (1.9 mmoles) in 25 ml methanol was debenzylated with H_2 and 0.3 g 5 % Pd on Al_2O_3 in a Parr hydrogenation apparatus for 4 h. After filtration with Celite, the solvent was evaporated yielding 0.21 g (54 %) blue crystals, m.p. 169—170°. Recrystallization from methanol did not change the melting point. Reported [8] m.p. 168—170° and [1] 173—176°. Spec. activity 261 μC/mM.

4-Hydroxytryptamine-2'-^{14}C (VII) Fig. 1.

4-Benzyloxygramine [9] was quaternized with dimethylsulfate to 4-benzyloxygramine methosulfate (V) according to Schöpf and Thesing.[10] Yield 90 %; m.p. 144—145°. Reported [11] m.p. 143—144°.

4-Benzyloxyindolyl-(3)-acetonitrile-2'-^{14}C (VI). 2.7 g V (6.65 mmoles) and 0.22 g $Na^{14}CN$ (0.4 mC; 4.43 mmoles) was dissolved in 25 ml water in an ampoule, which was sealed and heated on a steam bath for one hour. The nitrile separated as an oil. The ampoule was cooled and the mixture extracted with chloroform. The extract was dried over Na_2SO_4 and the solvent evaporated, affording 0.9 g (53 %) pale yellow oil, which was used in the next step without purification. IR: 2250 cm^{-1} (C≡N).

4-Benzyloxytryptamine-2'-^{14}C. 0.9 g VI was reduced with 0.3 g $LiAlH_4$ as described for II. Yield 0.51 g (57 %), oil, which did not crystallize. Hydrogen oxalate: m.p. 114—116°. Reported [9] m.p. 117—120°.

4-Hydroxytryptamine-2'-^{14}C (VII). The previous compound was debenzylated as described for III to give 84 % of an oil that did not crystallize. Oxalate m.p. 261—264°. Reported [9] m.p. 269—270°. Spec. activity 87 μC/mmole as base.

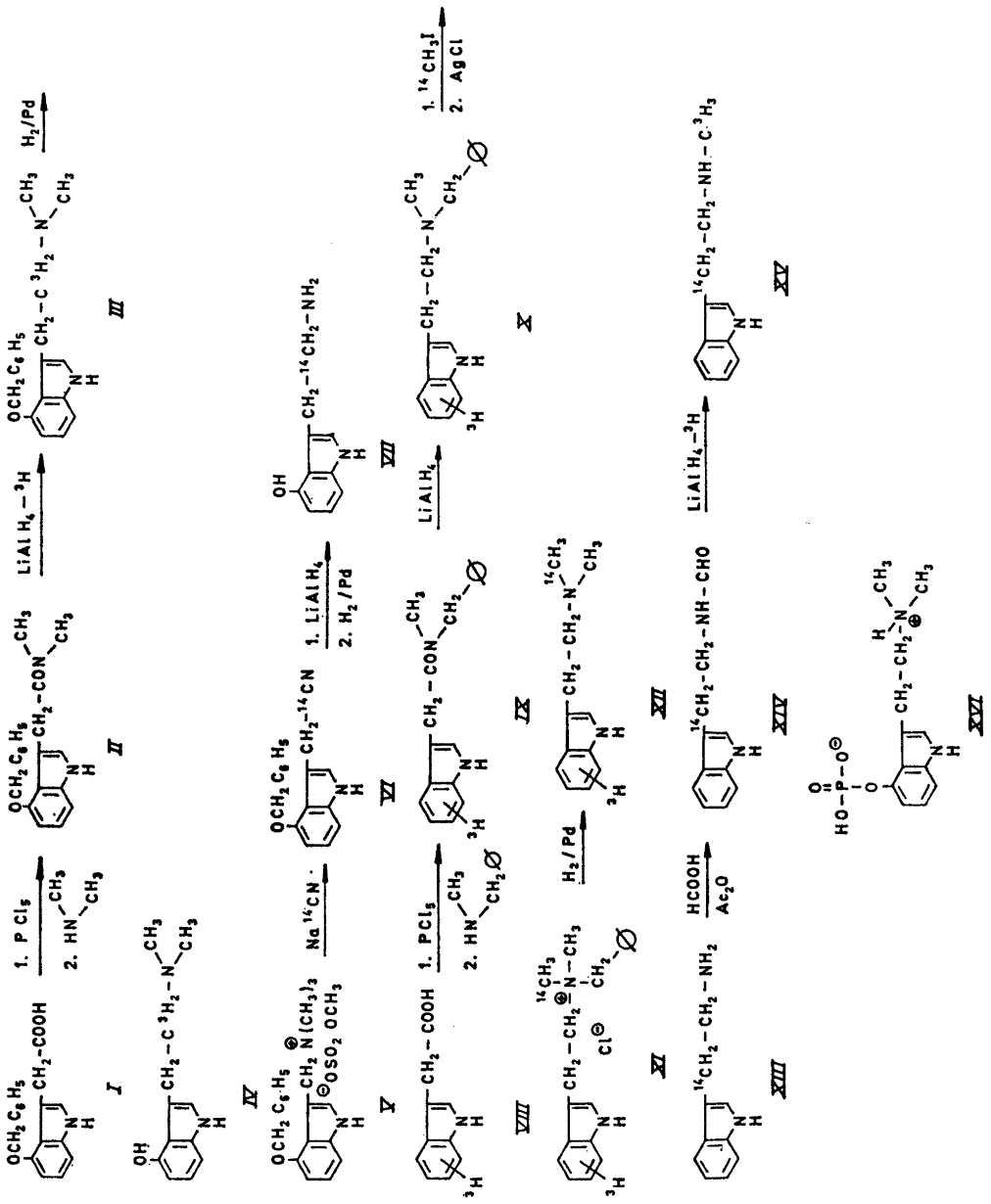

Fig. 1. Synthesis of ^{14}C- and ^{3}H-labelled precursors.

N,N-Dimethyltryptamine-2'-^{14}C.

3-Indoleacetonitrile-2'-^{14}C was prepared from 4.5 g (15 mmoles) gramine methosulfate[10] and 0.5 g Na^{14}CN (0.25 mC; 10 mmoles) in 25 ml water as described for VI. Yield 1.2 g (77 %) of an oil, which was used in the next step without purification. IR: 2250 cm^{-1} (C≡N).

3-Indoleacetic acid-2'-^{14}C was prepared from the nitrile by alkaline hydrolysis.[12] Yield 70 %; m.p. 162—165° (decomp.), reported[11] m.p. 165—166° (decomp.).

3-(N,N-Dimethylcarbamoylmethyl)indole-2'-^{14}C was prepared from indoleacetic acid-2'-^{14}C by the method used for II affording an oil (yield 87 %) which was used in the next step without purification.

N,N-*Dimethyltryptamine-^{14}2'-C* = *3-(2-dimethylaminoethyl)indole-^{14}C*. 0.25 g of the amide was reduced with 0.2 g $LiAlH_4$ as described for II. Yield 0.147 g (62 %) base as a pale yellow oil. Its hydrochloride melted at 159—163° after recrystallization from ethanol and ether. Reported[13] m.p. 166—167.5°. Spec. activity 75 μC/mmole.

N,N-Dimethyltryptamine(-indole-^{3}H-*N*-methyl-^{14}C) (XII). Fig. 1.

3-(N-Benzyl-N-methylcarbamoylmethyl)indole (IX) was prepared from (1.6 mC) 3-indoleacetic acid-indole-^{3}H (VIII) and *N*-benzyl-methylamine as described for II. Yield 71 %; m.p. 149—150°.

1.0 g of IX was reduced as described for II with $LiAlH_4$ in tetrahydrofuran to yield (86 %; 0.97 mC) *N-benzyl-N-methyltryptamine-^{3}H* (X).

0.80 g of X dissolved in 15 ml ethylacetate was quaternized with 0.5 g methyl iodide (0.1 mC) in an ampoule at 100° for one hour.[8] The crystalline deposit was washed with ethylacetate and then dissolved in 50 ml of 50 % aqueous methanol. This solution was treated with freshly prepared AgCl [8] to convert the iodide to the chloride (XI).

A solution of XI in 25 ml dry methanol was debenzylated as described for III. After column chromatography 0.31 g of the pure, doubly labelled N,N-*dimethyltryptamine* (XII) was obtained with a spec. activity: ^{3}H 320 μC/mmole; ^{14}C 11.2 μC/mmole. m.p. 43—45°; reported[17] m.p. 48—49°.

N-Methyltryptamine-1'-^{14}C-*N*-methyl-^{3}H (XV). Fig. 1.

Tryptamine-1'-^{14}C (XIII) 0.21 g (0.02 mC; 1.3 mmole) was formylated with 0.18 ml formic acid in 0.41 ml acetic anhydride according to Stauffer.[14] The *N*-formyltryptamine (XIV) was reduced with 0.4 g $LiAlH_4$ in refluxing tetrahydrofuran for 12 h to yield 0.10 g (44 %) of *N-methyltryptamine-1'-^{14}C* (XV). Spec. activity 8.0 μC/mmole.

N-Formyltryptamine (0.24 g) was similarly reduced with $LiAlH_4$-^{3}H (0.10 g; 0.5 mC) to *N*-methyltryptamine-*N*-methyl-^{3}H. Yield 0.10 g (47 %). Spec. activity 66.0 μC/mmole.

N-*Methyltryptamine-(1'-^{14}C-N-methyl-^{3}H)* = *3-(2-methylaminoethyl)-indole-^{14}C-^{3}H* (XV) was obtained by mixing ^{14}C-labelled with ^{3}H-labelled compound.

Other compounds

DL-*Tryptophan-2'-^{3}H*. Gramine 1.74 g (10 mmoles), 2.60 g (12 mmoles) ethyl acetamidomalonate and 0.20 g powdered NaOH in 30 ml dry toluene [15] were refluxed for 14 h under N_2. The hot solution was filtered and upon standing overnight, 2.52 g (73 %) of ethyl α-acetamido-α-carbethoxy-β-(3-indole)-propionate was deposited. This compound (2.0 g) was saponified and decarboxylated [16] in tritiated water (25 mC) to DL-tryptophan) (alanine-2'-^{3}H) which was crystallized from aqueous ethanol. Yield 0.23 g (19 %); m.p. 279—281°. Reported[16] m.p. 281—285°. Spec. activity 293 μC/mmole.

Generally tritium labelled compounds. 0.1 g of the aromatic compound to be tritiated was dissolved in 0.5—1 ml tritiated water (200—500 mC/ml) in an ampoule and the mixture was saturated with HCl gas. The sealed ampoule was treated on a steam bath for one hour. The evaporated residue was repeatedly dissolved in methanol to exchange

labile hydrogens. This acid catalyzed exchange procedure will exchange predominantly the hydrogens of the indole nucleus.[16] The crude tritiated product was purified by preparative paper [5] or column [6] chromatography. The purified amines were dissolved in dilute acid together with 2—4 times the amount of carrier material. After the addition of alkali, the amines were extracted with benzene and crystallized as base or hydrochloride.

4-Benzyloxytryptophan was tritiated in about 1 N HCl-solution to minimize the destruction and debenzylated as described for III. It was purified in system BAW.[5] The tritiation of 3-indoleacetic acid (0.16 g in 1.5 ml of 50% aqueous methanol; 140 mC) gave a particularly pure and highly labelled compound.

Specific activities: Tryptamine-^{3}H: 43.5 μC/mmole; *N*-methyltryptamine-^{3}H: 810 μC/mmole; *N*,*N*-dimethyltryptamine-^{3}H: 161 μC/mmole, DL-4-hydroxytryptophan-^{3}H: 96.1 μC/mmole; 3-indoleacetic acid: 1.8 mC/mmole.

Other conditions

Culture conditions etc. The method for submerged biosynthesis of psilocybin by *P. cubensis*, introduction of precursors and the isolation, purification and recrystallization of psilocybin are described previously as well as different assay procedures.[5]

Psilocybin was isolated as described [5] by methanol extraction, ion exchange technique and finally preparative paper chromatography. The following criteria [5] were used for determination of incorporation of label from a precursor into derived psilocybin: by TLC (in MAW), by paper chromatography (in IAW and BAW), and by electrophoresis followed by scanning, it was shown that the radioactivity was firmly associated with the isolated psilocybin and finally after the addition of carrier psilocybin (7—10 mg), psilocybin was crystallized from hot methanol to constant spec. activity.

To minimize the individual differences in incorporation rates between different cultures, a number of experiments were carried out (Table 1), where two different precursors, one ^{3}H-labelled, the other ^{14}C-labelled, were introduced into the same culture.

Chromatographic methods. The chromatographic systems have been described earlier.[5] Paper chromatography on Whatman 3MM paper with the following solvent systems BAW: butanol-acetic acid-water (4:1:5); IAW: isopropanol-conc. NH_4OH-water (8:1:1). TLC on Silica Gel G in system MAW: methanol-acetic acid-water (75:10:15). 4-Hydroxytryptamine which was not included in the previous paper [5] has the following R_F-values: in system BAW: 0.85; in system MAW: 0.75; and has a similar mobility as psilocin in the electrophoretic system. Psilocybin from 4-hydroxytryptophan-^{3}H fed cultures was isolated after purification first in system IAW and then in system BAW.[5]

Uptake of precursors from medium. About 1.0 mg of labelled precursor was introduced into each of two cultures. At zero time and then at different time intervals (Fig. 2) samples were withdrawn aseptically and the radioactivity in 500 μl of the medium was determined. To determine the percentage of radioactivity still present as the originally introduced compound, an aliquot of the medium, if necessary after concentration by lyophilization, was separated in systems MAW (Eastman chromatogram sheet) or BAW. Strips of the chromatogram were counted in a liquid scintillator.

RESULTS AND DISCUSSION

Labelled precursors. Psilocin-^{3}H, 4-hydroxytryptamine-^{14}C, *N*,*N*-dimethyltryptamine-^{14}C and -^{14}C-^{3}H, *N*-methyltryptamine-^{14}C-^{3}H, and DL-tryptophan-^{3}H were synthesized by appropriate modifications of known procedures [8,9,12,14–16] as illustrated in Fig. 1, to give suitably, singly or doubly, specifically labelled compounds. *N*-Methyltryptamine-*N*-methyl-^{3}H was conveniently prepared from tryptamine by formylation and reduction of the *N*-formyltryptamine with tritiated $LiAlH_4$. Ring tritiated *N*,*N*-dimethyltryptamine-^{3}H, *N*-methyltryptamine-^{3}H, tryptamine-^{3}H, DL-4-hydroxytryptophan-^{3}H (as the benzyloxy compound), and 3-indoleacetic acid-^{3}H were obtained by acid catalyzed exchange in tritiated water.

Table 1. Incorporation of labelled precursors into psilocybin.

Expt. No.	Precursor introduced	D.p.m. × 10^{-6}	Mg	Precursor incorporated into psilocybin, %; (mg psilocybin formed)		Spec. act. of psilocybin μC/mmole		Dilution [a]
28	L-Tryptophan-^{3}H [b]	111	0.3	0.86	(0.48)	^{3}H	262	132
	Tryptamine-^{14}C	11.1	0.3	2.06		^{14}C	75.7	33
29	L-Tryptophan-^{3}H	111	0.3	0.88	(0.90)	^{3}H	139	244
	Tryptamine-^{14}C	11.1	0.3	2.56		^{14}C	33.3	74
36 [c]	DL-4-OH-Tryptophan-^{3}H	23.7	25	0	(0.62)	^{3}H	0	> 500
	DL-Tryptophan-^{14}C	5.55	20	0.86		^{14}C	9.62	2.7
37	DL-4-OH-Tryptophan-^{3}H	23.7	25	0	(1.43)	^{3}H	0	> 500
	DL-Tryptophan-^{14}C	5.55	20	1.49		^{14}C	7.38	3.5
38	*N*-Methyltryptamine-^{3}H	35.2	3.4	14.8	(1.64)	406		2.0
42	*N*-Methyltryptamine-^{3}H	32.0	3.1	9.63	(0.58)	679		1.2
40	*N*-Methyltryptamine-^{3}H	178	10	0.91	(1.72)	^{3}H	143	5.7
	Tryptamine-^{14}C	11.1	11	1.09		^{14}C	7.60	9.7
11	*N,N*-Dimethyltryptamine-^{14}C [b]	3.22	3.6	0.15	(0.25)		2.30	31
15	*N,N*-Dimethyltryptamine-^{14}C	4.25	4.8	0.17	(0.43)		2.20	34
20	4-OH-Tryptamine-^{14}C	4.05	5.8	3.73	(3.44)		5.63	9.8
26	4-OH-Tryptamine-^{14}C	3.20	4.6	0.98	(1.14)		3.52	16
10	Psilocin-^{3}H	17.0	6.0	2.86	(2.60)		23.9	11
19	Psilocin-^{3}H	18.5	6.5	8.48	(4.85)		41.4	6.3
21	Psilocin-^{3}H	19.9	7.0	0.71		^{3}H	27.2	9.6
	N,N-Dimethyltryptamine-^{14}C	5.57	6.3	0.17	(16.0)	^{14}C	0.75	100
27	Psilocin-^{3}H	11.6	4.1	0.95		^{3}H	34.4	7.6
	N,N-Dimethyltryptamine-^{14}C	3.72	4.2	0.09	(0.41)	^{14}C	1.00	75

[a] Spec. act. of precursor/spec. act. of psilocybin.
[b] For location of label, see Experimental section.
[c] Signifies that two differently labelled precursors were introduced into the same culture.

Biosynthesis of psilocybin. A number of alkaloids (*cf.* Ref. 22) contain an indole nucleus hydroxylated in position 4 (*e.g.* venenatine,[5] psilocin), in position 5 (*e.g.* ibogaine, eserine), in position 6 (*e.g.* reserpine, vindoline), in position 7

(*e.g.* aspidospermine, vallesine) or in more than one positon. Several indole alkaloids have been demonstrated to be derived from tryptophan.[23] However, one of the still lacking, interesting points in our knowledge is whether this hydroxyl group is introduced into tryptophan or if the hydroxylation of the indole nucleus occurs during some later stage as a result of secondary conversions of the formed alkaloid skeleton.

Psilocybin is derived from tryptophan [4,5] but it is not known in which specific sequence, if any, the necessary modifying reactions (decarboxylation, *N*-methylation, 4-hydroxylation, and *O*-phosphorylation) occur. For the biosynthesis of psilocin, one could suggest analogies in the biological formation of serotonin and gramine. The latter compound is derived from tryptophan by a dealkylation to 3-aminomethylindole followed by step-wise *N*-methylation.[19,20] Serotonin is biosynthesized, as numerous investigations have shown, from tryptophan *via* 5-hydroxytryptophan,[21] thus, initially, by a hydroxylation of tryptophan followed by a decarboxylation.

However, the results in Table 1 show that 4-hydroxytryptophan which so far has not been encountered in nature, in contrast to tryptophan does not function as a precursor of psilocybin. Tryptamine, which is readily formed from tryptophan by *P. cubensis*,[6] serves as a better precursor of psilocybin than tryptophan. If tryptamine is methylated to *N*-methyltryptamine, this is an even better progenitor of psilocybin with incorporations showing that more than half of the psilocybin was derived from the introduced labelled precursor (Expts. Nos. 38, 42). The corresponding dimethyl derivative *N*,*N*-dimethyltryptamine may, however, be considered as a poor precursor as judged from the incorporation percentages. These are decidedly lower than for, *e.g.*, tryptophan and definitely not in agreement with *N*,*N*-dimethyltryptamine standing biogenetically closer to psilocybin than does tryptophan.

Nevertheless, with other factors taken into account the low incorporation percentages for *N*,*N*-dimethyltryptamine does not make it an unlikely intermediate. If the "dilution factors" for this compound and for psilocin (Expts. Nos. 21, 27) are compared, it is evident that psilocin is about ten times more effectively incorporated into psilocybin. This large difference led us to investigate the up-take of different precursors from the culture medium by the fungus with the striking differences shown in Fig. 2. At the time when the labelled compounds are administered, 12—15 % of the culture volume is taken up by the cells. This means that *N*,*N*-dimethyltryptamine (Fig. 2) actually to some extent is prevented from entering the cells, since in this and two other experiments at any time no more than 7 % of this labelled material, usually much less, had disappeared from the liquid medium. This poor absorption of the precursor was verified by the low activity present in the methanol extract of the fungus.

The activity of administered non-hydroxylated indoles remained in the medium, as shown by TLC, still mainly (not less than 74 %) as the originally administered compounds after 44 h. The pH of the medium was, as could be expected, crucial for the stability of the 4-hydroxylated indoles. The pH of the culture medium is at start 5.2 and is then slightly decreasing to 4.8—5.0 between the 4th and 10th day and increases then slowly. This means, however, that during the experiments the 4-hydroxylated indoles have a reasonable

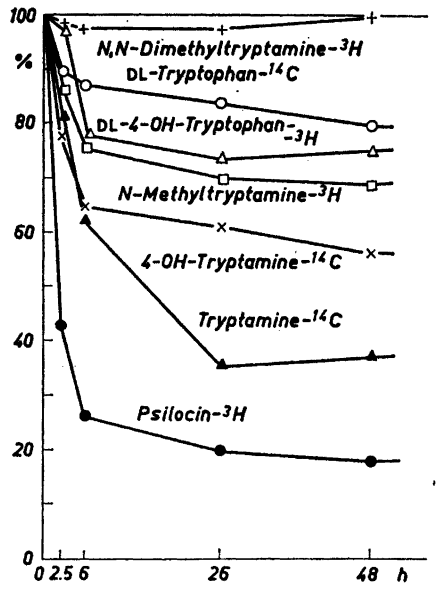

Fig. 2. Up-take of precursors from medium by *P. cubensis*. Per cent of introduced radioactivity remaining in medium.

stability. Thus psilocin remained unchanged to 63 % after 44 h in a medium of pH 5.2 but was quickly destroyed at a pH over 7.

The curves in Fig. 2 show, as expected, that the up-take is most rapid at the beginning, reaching a maximum after about 24 h followed by a slow, small (less than 10 %) release of radioactivity back into the medium over the next 96 h. If the points on the initial parts of the curves are used to get a rough estimate of the clearance of the precursors from the medium, the following approximate half-lives are obtained for: psilocin = 2 h, tryptamine = 8 h, tryptophan = 16 h, and *N*,*N*-dimethyltryptamine over 40 h.

The above data would suggest the following sequence (Fig. 3) for the formation of psilocybin:

tryptophan ⟶ tryptamine ⟶ *N*-methyltryptamine ⟶ *N*,*N*-dimethyltryptamine ⟶ psilocin ⟶ psilocybin.

Experiments with doubly labelled *N*-methyltryptamine and *N*,*N*-dimethyltryptamine are in progress to ensure that these compounds are converted *in toto* to psilocybin without previous demethylation.[24]

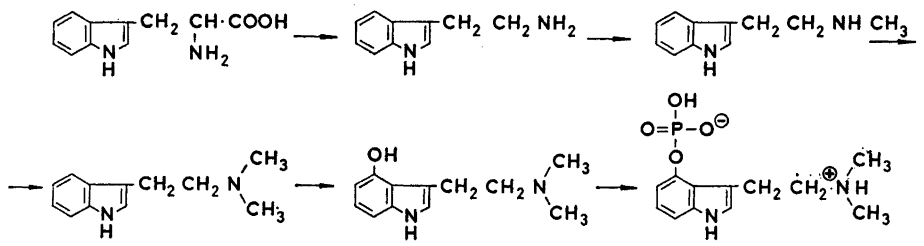

Fig. 3. A possible pathway of psilocybin biosynthesis as indicated by present experiments.

The data in Table 1 also indicate that the fungus can utilize another path to psilocybin since also 4-hydroxytryptamine is well incorporated into psilocybin. Here, the hydroxyl group occurs already at the tryptamine-stage thus before the *N*-methylation steps. It may be noted that 4-hydroxytryptamine apparently is a metabolite of 4-hydroxytryptophan in animals.[21] When 4-hydroxytryptamine was introduced into the culture, this occasionally led to the formation also of small amounts of one or two "psilocybin-like" compounds which possibly may have been phosphoric esters of 4-hydroxytryptamine and its *N*-methyl derivative, the recently isolated baeocystin.[25]

Thus it appears the *P. cubensis* can utilize two pathways to psilocybin. Further experiments with *N*-methyl-4-hydroxytryptamine-^{14}C-^{3}H and doubly labelled tryptamines as well as experiments on "apparent intermediates" which are now in progress [6] are required to show if the fungus normally uses more than one pathway from tryptophan to psilocybin. So far, only tryptamine and psilocin have been isolated from the fungus as "apparent intermediates" in this process.

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REFERENCES

1. Hofmann, A., Heim, R., Brack, A., Kobel, H., Frey, A., Ott, H., Petrzilka, T. and Troxler, F. *Helv. Chim. Acta* **42** (1959) 1557.
2. Catalfomo, P. and Tyler, Jr., V. E. *Loydia* **27** (1964) 53.
3. Leung, A. Y., Smith, A. H. and Paul, A. G. *J. Pharm. Sci.* **54** (1965) 1576.
4. Brack, A., Hofmann, A., Kalberer, F., Kobel, H. and Rutschmann, J. *J. Arch. Pharm.* **294** (1961) 230.
5. Agurell, S., Blomkvist, S. and Catalfomo, P. *Acta Pharm. Suecica* **3** (1966) 37.
6. Agurell, S. and Nilsson, L. *Acta Chem. Scand. To be published.*
7. Agurell, S. *Acta Pharm. Suecica* **3** (1966) 11.
8. Kalberer, F., Kreis, W. and Rutschmann, J. *Biochem. Pharmacol.* **11** (1962) 261.
9. Stoll, A., Troxler, F., Feyer, J. and Hofmann, A. *Helv. Chim. Acta* **38** (1955) 1452.
10. Schöpf, C. and Thesing, J. *Angew. Chem.* **63** (1951) 377.
11. Floss, H. G. and Mothes, U. *Z. Naturforsch.* **18b** (1963) 866.
12. Thesing, J. and Shülde, F. *Chem. Ber.* **85** (1952) 324.
13. Vitali, T. and Messini, F. *Boll. Sci. Fac. Chim. Ind. Bologna* **17** (1959) 84.
14. Stauffer, R. *Helv. Chim. Acta* **49** (1966) 1199.
15. Howe, E. E., Zambito, A. J., Snyder, K. R. and Tishler, M. *J. Am. Chem. Soc.* **67** (1945) 38.
16. Barton, D. H. R., Kirby, G. W., Prager, R. H. and Wilson, E. M. *J. Chem. Soc.* **1965** 3990.
17. Budendorf, K. and Walk, A. *Arch. Pharm.* **294** (1961) 484.
18. Snyder, H. R. and Smith, C. W. *J. Am. Chem. Soc.* **66** (1944) 350.
19. O'Donovan, D. and Leete, E. *J. Am. Chem. Soc.* **85** (1963) 461.
20. Gower, B. G. and Leete, E. *J. Am. Chem. Soc.* **85** (1963) 3683.
21. Harborne, J. B., (Ed.), *Biochemistry of phenolic compounds*, Academic, London and New York 1962, pp. 230–234.
22. Boit, H. G. *Ergebnisse der Alkaloid Chemie bis 1960*, Akademie-Verlag, Berlin 1961.
23. Stolle, K., Gröger, D. and Mothes, K. *Chem. Ind. (London)* **1965** 2065.
24. Floss, H. G. and Gröger, D. *Z. Naturforsch.* **19b** (1964) 393.
25. Leung, A. Y. and Paul, A. G. *J. Pharm. Sci.* **56** (1967) 146.

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