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THE FATE OF PSILOCIN IN THE RAT

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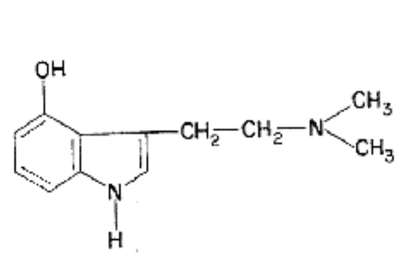
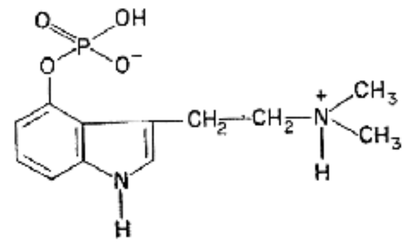
Abstract—The synthesis of ^{14}C-psilocin labelled in two different positions is described. The resorption, distribution, excretion and metabolism of psilocin in the rat was studied quantitatively with the labelled compounds.

INTRODUCTION

PSILOCIN (I) is one of the psychotomimetic substances isolated from the mushroom *Psilocybe mexicana*, the principal active compound found in the drug being its O-phosphorylated derivative psilocybin (II).[1–4]



I



II

The pharmacological actions of (I) and (II) are qualitatively and quantitatively so similar,[5, 6] that it may be assumed that the phosphoric ester group of II is very rapidly cleaved in the body and that the true active agent of the drug is in fact I. We have carried out a study of the fate of ^{14}C-labelled psilocin in the rat and believe that the results, except those concerning resorption, are valid for psilocybin also.

Two labelled forms of the substance were used. The first one carried the isotopic atom in position 2′ of the side chain (Ia), while the other was labelled in the N-methyl group (Ib). In Ia the labelled position proved to be metabolically stable since the amount of $^{14}CO_2$ in the expired air of animals treated with it corresponded to less than 1 per cent degradation in 48 hr. Ib was used for the study of oxidative demethylation and deamination.

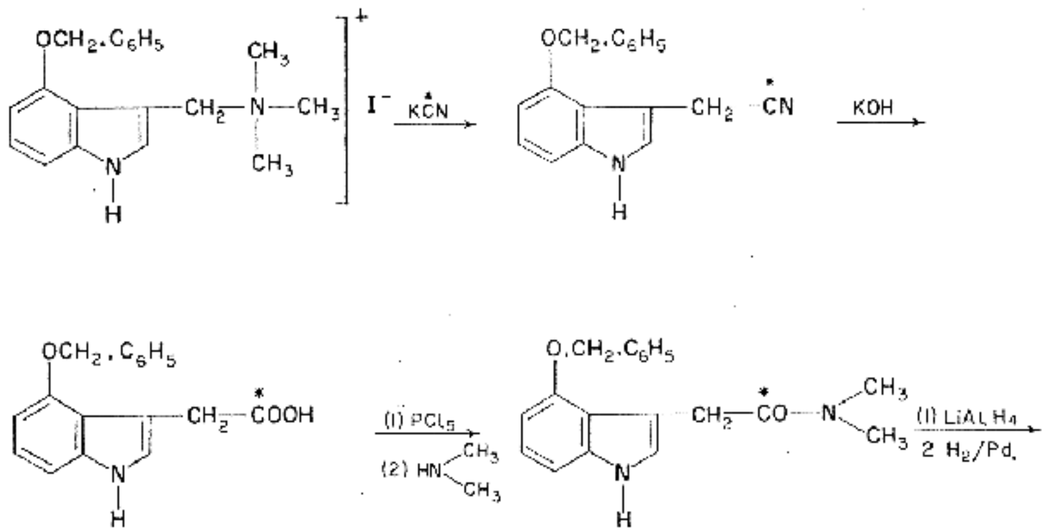
MATERIALS AND METHODS

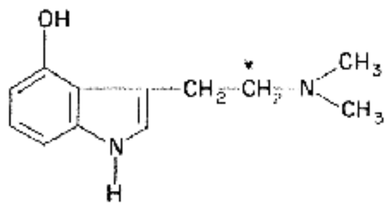
Synthesis of psilocin-2′-^{14}C (Ia)

For the synthesis of 4-benzyloxy-indole-acetic acid, methods described in Ref. 4 were adapted to the needs of radioactive work. The acid was transformed into the diethylamide according to the method described by Shaw and Wooley.[7]

One and a half grammes (3·5 m-mole) 4-benzyloxy-gramine-methoiodide[8] and 10 ml of an aqueous solution containing approximately 2 m-moles of ^{14}KCN (5 mc) were

sealed into a cooled ampoule and then heated for 1 hr in a boiling water bath. The ampoule was cooled in ice–salt mixture, opened and 0·5 ml of 1 N NaOH was added. The resulting product was extracted with chloroform and the extracts, after washing with sodium bicarbonate and removal of water, were evaporated to dryness. The residues from two identical runs were dissolved in chloroform and the solution filtered through 40 g of alumina. The substance was subsequently eluted with 500 ml of chloroform. A weight of 1·55 g of crude nitrile was obtained by evaporation of the eluate.





Ia

The nitrile was saponified by refluxing with 3 g KOH in 9 ml ethanol and 6 ml water with stirring for 20 hr. After dilution with water, neutral substances were extracted with chloroform. The aqueous solution was then acidified with 2 N HCl while being cooled and extracted with chloroform–*iso*propanol 2:1. The extraction was repeated after addition of 150 mg of inactive 4-benzyloxy-indole-acetic acid as carrier. On evaporation of the extracts, 720 mg of crude acid, which were used for the following step without purification, were obtained. The acid was suspended in 25 ml of absolute ether and stirred at 0 °C with 600 mg of phosphorus pentachloride until a clear solution was obtained. This solution was cooled while 30 ml of a 10 per cent solution of dimethylamine in absolute ether were added and stirring was then continued for 30 min. After adding 1 N sodium-bicarbonate solution, the product formed was extracted with chloroform and the residue of the extract was crystallized

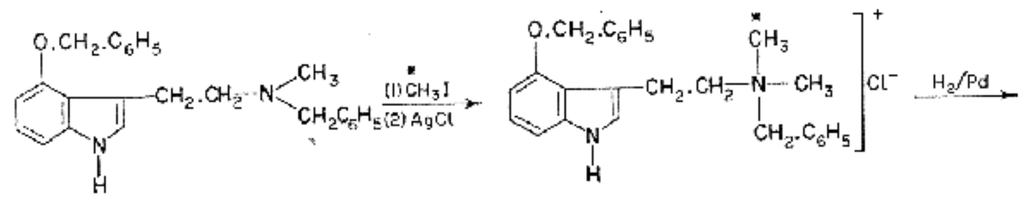
from benzene. The yield of 4-benzyloxy-indole-dimethyl-acetamide (m.p. 175–178 °C*), was 743 mg.

The amide was dissolved in 20 ml of absolute tetrahydrofuran and the solution was added, with stirring and cooling, to 350 mg $LiAlH_4$ suspended in 10 ml of the same solvent. The mixture was stirred (moisture not being allowed access) for 45 min at 40 °C, the excess of $LiAlH_4$ cautiously removed with water, 20 per cent NaOH added and the reduction product extracted with ether. The residue of the ether solution was recrystallized from ether-petroleum ether, yielding 648 mg of benzyl-psilocin, (m.p. 120–121 °C).

The benzyl-psilocin was debenzylated catalytically in methanol with a palladium catalyst (5 per cent on alumina). Within 7 hr the theoretical amount of H_2 was absorbed at ambient temperature and pressure. The catalyst was then filtered off, the solution concentrated to a few millilitres and the psilocin finally crystallized by adding an equal volume of benzene. The yield of recrystallized product (m.p. 168–170°), was 355 mg; the specific activity was 1·60 mc/m-mole. From the mother liquor a second crop of pure product of lower specific activity could be recovered after addition of carrier-psilocin. Both fractions were radiochemically pure as shown in the chromatographic system *n*-butanol–acetic acid–water 4:1:5. Thirty-one per cent of the ^{14}C introduced into the synthesis as $Ba^{14}CO_3$ (for the preparation of the cyanide) was recovered as radioactive psilocin.

Synthesis of N-*methyl*-^{14}C-*psilocin* (Ib)

The synthesis was effected according to the following reaction sequence:



Ib

550 mg (1·48 m-mole) of 4-benzyloxy-N-methyl-N-benzyl-tryptamine† were sealed into a small glass tube together with 10 ml of ethyl acetate and 204 mg (1·44 m-mole, 1 mc) of ^{14}C-methyl iodide and heated for 30 min in a boiling water bath. After opening the tube, the crystalline precipitate of the methoiodide was washed by decantation with ethyl acetate and then dissolved in 50 ml of 50 per cent methanol. The solution was shaken for 30 min with silver chloride freshly prepared from 2 mg of nitrate, the solids were filtered off over hyflo, and the solution was evaporated *in vacuo*. On

* Melting points are determined on a block and are not corrected.

† F. Troxler, unpublished. The compound was prepared according to methods described in Ref. 4.

crystallization of the residue from methanol–ethyl acetate, 591 mg (97 per cent) of the methochloride were obtained. The compound was catalytically debenzylated over palladium catalyst in methanol. Within 3 hr the theoretical amount of H_2 was taken up. After filtration the solution was added to 150 ml of molar sodium bicarbonate and extracted several times with ether. The residue of the extract was taken up in 5 ml of chloroform containing 1·5 per cent of methanol and the solution filtered through 4 g of alumina which was then washed with 100 ml of the same solvent. After evaporation of the filtrate, 245 mg (86 per cent) of ^{14}C-psilocin were obtained by crystallization from benzene–*cyclo*hexane. After recrystallization, 200 mg of a product with a melting point of 167–169 °C and a specific activity of 0·66 mc/m-mole were recovered. This substance proved to be radiochemically pure in thin layer chromatography on silica using *n*-butanol–acetic acid–water 4:2:5.

Determination of ^{14}C

Pure substances and dried tissues were analysed by our routine laboratory method comprising wet combustion of 3–7 mg and counting of the $^{14}CO_2$ evolved in proportional gas counters.[9, 10] Tissues were weighed after dissection dried in high vacuum over night at room temperature, weighed again and ground. Suitable aliquots of solutions and excreta were evaporated under vacuum in an oxidation vessel and then

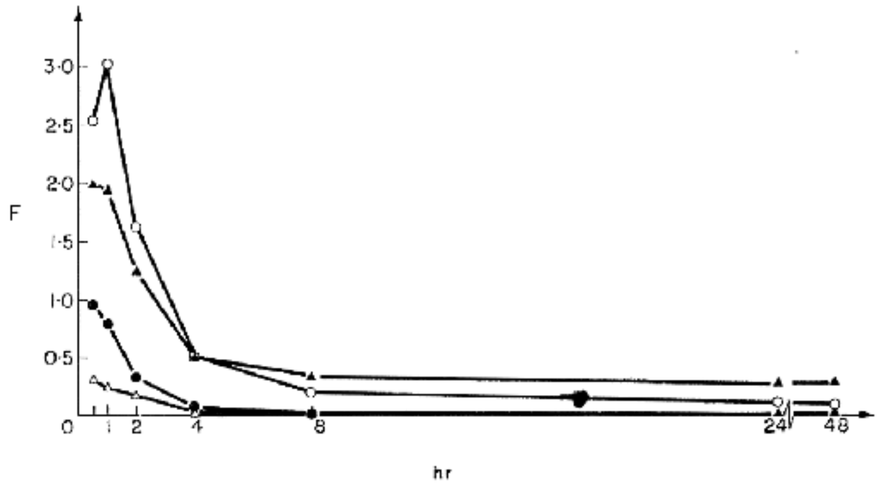


FIG. 1. Distribution of ^{14}C-psilocin in the kidneys ○——○, liver ▲——▲, brain ●——●, blood △——△.

analysed. If necessary, carrier-carbon was added in the form of succinic acid. The radioactivity level of the tissue is expressed by a factor F, defined as follows:

$$F = \frac{\text{counts/min per unit weight} \times \text{weight of animal}}{\text{counts/min administered}}$$

F values of less than 1·1 and more than 1 denote, respectively, low, uniform and high levels of radioactivity. Radiochromatograms were scanned in an automatic device constructed in our laboratory, using a windowless Geiger flow counter.

Animals and dosage

In all experiments male albino rats (Glaxostrain) of 180–230 g were used. A standard dose of 10 mg/kg of psilocin was injected intravenously in the caudal vein or applied orally by stomach tube.

RESULTS AND DISCUSSION

Resorption

The standard oral dose of Ia was applied to four pairs of rats which then were killed under ether narcosis by exsanguination through the vena cava after ½, 1, 2 and 4 hr, respectively. The gastro-intestinal tract (GIT) was prepared from cardia to rectum, taking care to remove as much as possible of the mesenterium and connective tissue, etc., and homogenized first in a blender and then in a Potter homogenizer. Radioactivity was determined on aliquot samples. After ½ hr, 75 per cent and after 1 hr, 60 per cent of the radioactivity was found in the GIT; the activity remaining in the GIT approached the value 50 per cent asymptotically during the next 3 hr. As the excretion of radioactive products via the bile (Fig. 2) could at best amount to 14 per cent of the quantity introduced into the circulation, i.e. 7 per cent of the dose administered, the results indicate that the rat is able to absorb slightly more than 50 per cent of the orally applied psilocin.

Distribution

The data on the distribution of Ia in the organs of the rat after intravenous injection are summarized in Table 1. In addition, the values for blood, brain, liver and kidney are presented graphically in Fig. 1.

TABLE 1. DISTRIBUTION OF RADIOACTIVE MATERIAL IN THE ORGANS

Organ	Factor F						
	½ hr	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr
Blood	0·31	0·25	0·19	0·04	0·04	0	0
Colon	0·53	0·51	0·37	0·18	0·12	0·05	0·03
Small intestine	1·46	0·91	0·48	0·10	0·07	0·03	0·02
Brain	0·97	0·80	0·34	0·09	0·02	0	0
Skin	1·14	0·95	0·66	0·22	0·14	0·13	0·05
Heart	0·58	0·46	0·29	0·08	0·04	0·02	0·02
Testes	0·72	0·55	0·45	0·15	0·04	0	0
Bone	0·14	0·41	0·11	0·07	0·01	0	0·01
Bone marrow	1·83	1·53	1·04	0·24	0·15	0·12	0·02
Liver	2·00	1·95	1·25	0·52	0·37	0·29	0·29
Lung	1·28	1·09	0·73	0·26	0·10	0·02	0·04
Stomach	1·52	0·56	0·42	0·14	0·08	0·02	0
Spleen	1·47	0·93	0·69	0·24	0·16	0·11	0·11
Muscle	0·58	0·43	0·28	0·08	0·03	0	0
Epididymis	0·11	—	0·09	0·03	0·03	0	0
Adrenals	2·68	2·13	2·00	0·99	0·84	0·34	0·39
Kidneys	2·54	3·04	1·63	0·52	0·22	0·12	0·08
Pancreas	0·26	0·44	0·30	0·11	0·03	0·02	0
Salivary glands	2·45	2·00	1·54	0·47	0·11	0·02	0·02
Thyreoidea	1·14	0·63	0·45	0·12	0·05	0·02	0·02

Excretion

The data on excretion of radioactive material after intravenous administration of Ia to rats with bile fistula are summarized in Table 2 and Fig. 2. The corresponding values obtained with intact animals are shown in Table 3 and Fig. 3. After 24 hr the

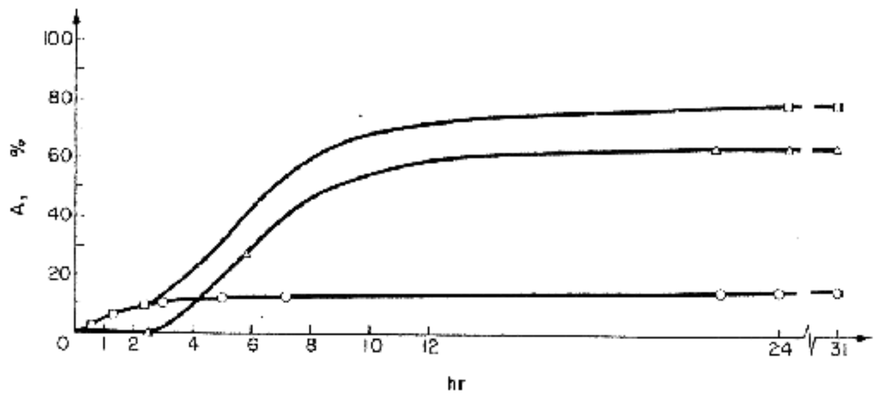


FIG. 2. Excretion of radioactive material in rats with bile fistula. ○——○ % excreted in the bile. △——△ % excreted in the urine. □——□ total.

TABLE 2. EXCRETION OF RADIOACTIVE MATERIAL IN RATS WITH BILE FISTULA

	% excreted in the bile	% excreted in the urine	Total	Time (hr)
Experiment 3	17·6	60·0	77·6	31
Experiment 4	12·4	67·5	79·9	31
Average	15·0	63·8	78·8	31

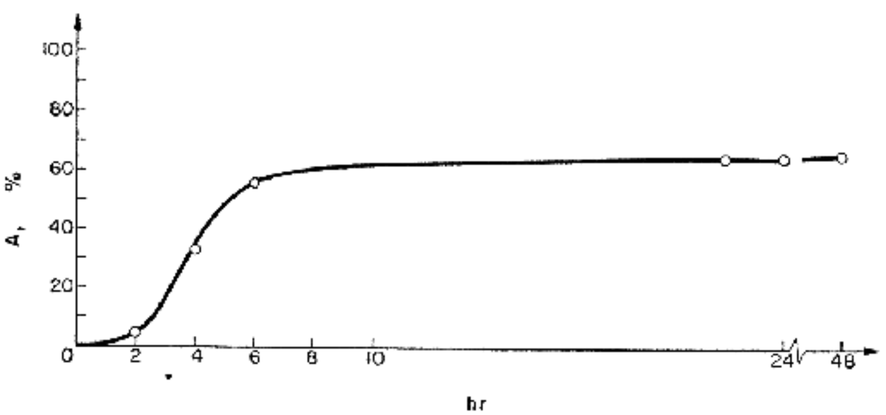


FIG. 3. Excretion of radioactive material in the urine of intact rats.

bodies of two rats from experiment 2 (Table 3) contained 7·1 and 6·6 per cent, respectively, of the applied radioactive material. Adding about 1 per cent for the substance lost by degradation of the side chain, about 94 per cent of the total activity is accounted for. The fate of the rest, i.e. 6 per cent is uncertain at the moment. Part of it might have been lost as CO_2 during sample preparation (drying of tissue, etc.) by decarboxylation of indole acetic acid derivatives.*

Metabolism

(a) *Demethylation and deamination.* After administration of N-methyl-^{14}C-psilocin (Ib), radioactive CO_2 appeared in the expired air as shown in Table 4. From these results it must be concluded that only 3·6 per cent of the psilocin can be degraded by the rat to indole acetic acid derivatives. If, alternatively, the formation of monomethylamino compounds is assumed, a maximum of 7 per cent of the injected drug can be involved.

TABLE 3. EXCRETION OF RADIOACTIVE MATERIAL IN INTACT RATS (EXPERIMENTS 1 AND 2)

Day	% excreted in the faeces	% excreted in the urine	Total per day	Accumulated total
1	21·3	62·0	83·3	
2	1·4	0·7	2·1	85·4
3	0·3	0·3	0·6	86·0
4	0·1	1·2	1·3	87·3
5–7	0·8	0·6	1·4	88·7

TABLE 4. EXCRETION OF $^{14}CO_2$ IN THE EXPIRED AIR

Time (hr) accumulated	Accumulated % $^{14}CO_2$ in expired air
½	0·28
1	0·63
2	1·05
4	1·57
8	2·17
24	2·69
48	3·57

(b) *Determination of unaltered psilocin in the urine by isotope dilution.* In radiochromatograms (Fig. 4) psilocin could be qualitatively identified in the urine. For its quantitative determination, the urine was collected over 24 hr in a weakly acid solution of 200 mg of inactive psilocin. The solution, containing 57 per cent of the applied active material, was made alkaline with $NaHCO_3$ and extracted three times with ether. Twenty-one per cent of the total activity was found in

* 4-Hydroxy-indole-acetic acid decomposes easily on sublimation at 0·05 mg Hg and 150 °C, 4-hydroxy-skatole being produced.
(Found: C, 73·0; H, 6·0; O, 11·0%. Calc. for C_9H_9ON: C, 73·4; H, 6·2; O, 10·9%).
In the infrared spectrum no COOH, CO or lactone bands are present.

the extract. From the residue of the ether solution, psilocin could be crystallized directly with methanol. The specific activity of the product did not change on further recrystallization; its radiochemical purity was proved chromatographically. From the specific activity of pure diluted substance, it was calculated that all the active material extracted from the urine with ether was in the form of unaltered psilocin:

$$\text{Found: } \frac{\text{counts/min per mg psilocin} \times 200 \times 100}{\text{counts/min of ether extract}} = 101 \text{ and } 102\%$$

(c) *Identification of* 4-*hydroxy-indole-acetic acid*. Urine collected over a period of 24 hr from rats treated with Ia was first extracted with ether to remove unaltered psilocin, acidified to pH 3 and then extracted with chloroform–methanol 9:1. In this extract, 4-HIAA was identified directly and as the corresponding ester after methylation with diazomethane by radiochromatography in two separate solvent systems (Fig. 5).

A quantitative determination of the metabolite by dilution analysis could not be effected because 4-HIAA is not crystalline, rather unstable and has not so far yielded any crystalline derivatives. From the results given in paragraph (a) and from the fact that no traces of demethylated amine have been found among the metabolites, it is concluded that the amount of expired $^{14}CO_2$ is representative for both demethylation and consecutive deamination and oxydation to 4-HIAA.

(d) *Other metabolites*. Psilocin and 4-HIAA account for approximately 40 per cent of the radioactivity found in the urine. The rest is present in hydrophilic form, only half of it being extracted from acid solution by butanol. The hydrophilic metabolites form one broad peak in paper radiochromatograms (Fig. 4, system *n*-butanol–acetic acid–water 4:1:5) with an R_f value of about 0·2. Up to now, we have not been able to definitely identify the products as glucuronides[11] (e.g. by treatment with glucuronidase and subsequent chromatography of the possible cleavage products, or by Williams' method.[12])

CONCLUSIONS

From the results presented it can be concluded that psilocin is absorbed to about 50 per cent by the gastrointestinal tract of the rat. Its distribution in the body is on the whole quite uniform, most of the organs and tissues showing an *F*-value of 0·5–1·0 after 30 min. The brain is not an exception ($F = 1$ after 30 min) in contrast to LSD, which reaches only very low brain levels.[13] The activities shown in the tissues (again including the brain) fall off in the course of 8 hr to low values. The only exceptions are the liver with $F = 0{\cdot}29$ and, interestingly enough, the adrenals with the relatively very high *F*-value of 0·39 after 48 hr. This result seems to deserve further attention.

The distribution values in relation to time are reflected in the excretion data. Psilocin and its metabolites are mainly excreted in the urine (approximately 65 per cent of the dose in 24 hr), whereas 15–20 per cent appears in the bile and faeces. Most of the products are excreted during the first 8 hr, but a considerable part (10–20 per

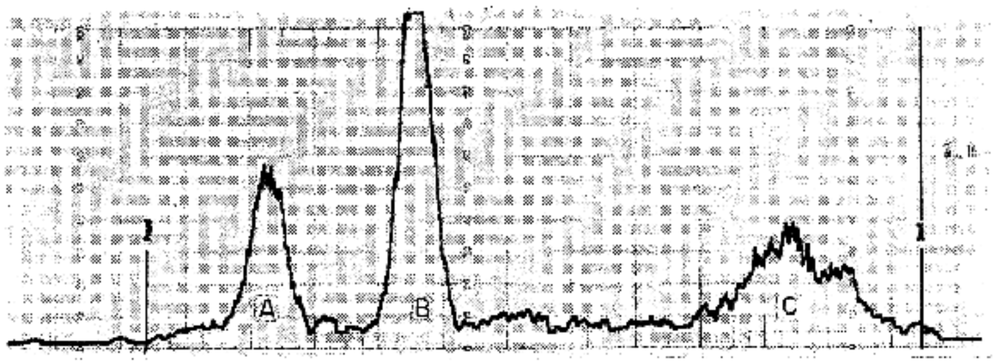


FIG. 4. Radiochromatogram of rat urine (system *n*-butanol–acetic acid–water 4:1:5). A · 4-HIAA. B · Psilocin. C · Unknown metabolites.

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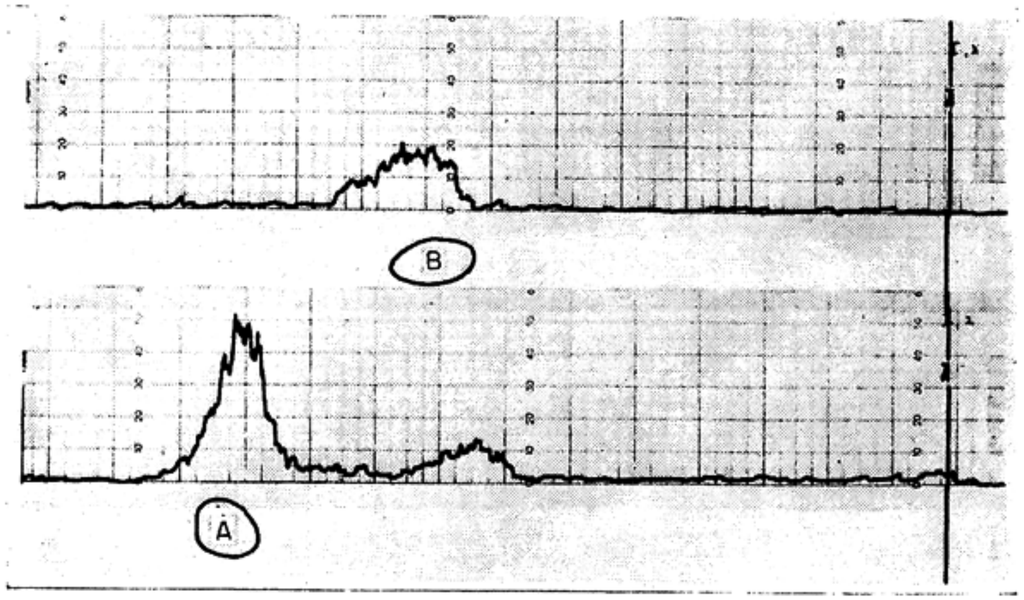

FIG. 5(1)

FIG. 5(2)

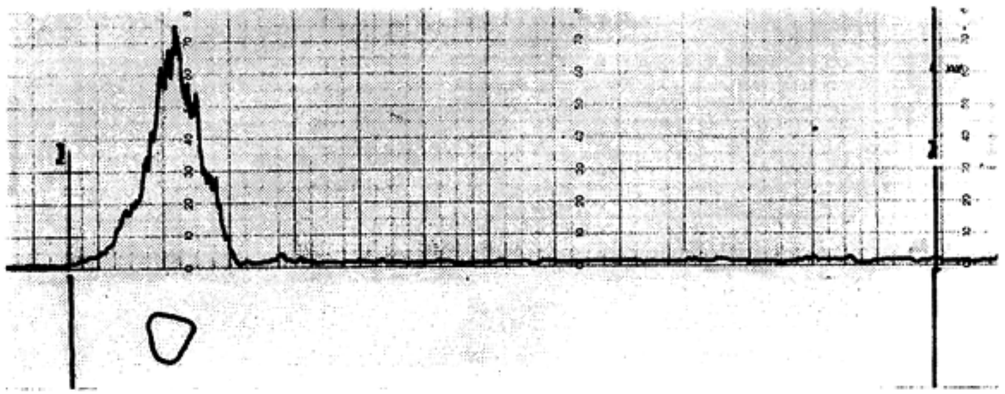

FIG. 5(3)

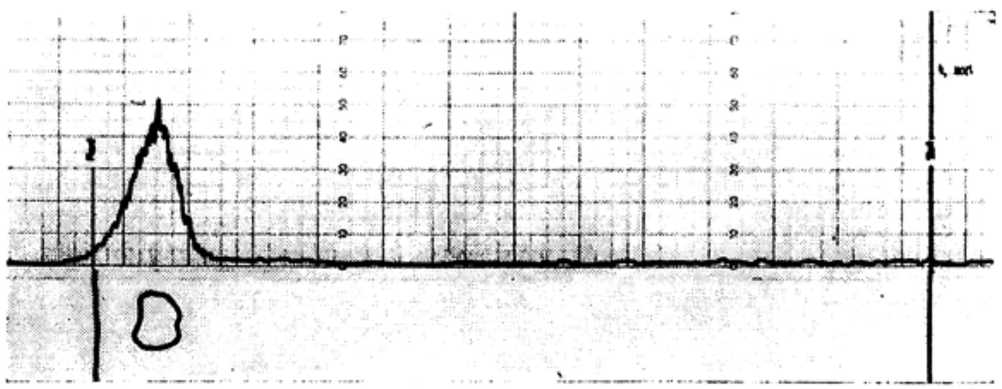

FIG. 5(4)

FIG. 5. Identification of 4-HIAA by radiochromatography.
(1) Chloroform methanol extract of acidified urine B: reference spot of 4-HIAA.
(2) After methylation with diazomethane A: reference spot of analogously treated 4-HIAA. System *n*-propanol–water 5:1.
(3) and (4) The same as (1) and (2) in the system *n*-butanol–acetic acid–water 4:1:5.

cent) is retained longer, so that even after 7 days, significant quantities of metabolites are found in the urine.

The qualitative and quantitative results of our experiments on the metabolism of psilocin indicate that the side chain of the drug is remarkably stable. The same seems to hold true for its isomers bufotenin and dimethyltryptamine and for psilocybin, although only qualitative results are presented in the literature.[11–15] Oxidative demethylation of the dimethyl-aminoethyl group plays only a minor role in the metabolism of psilocin. The resulting secondary and primary amines do not appear as end products, but are metabolized further to 4-HIAA. As shown in the experiments on the formation of $^{14}CO_2$ after administration of N-methyl-^{14}C-psilocin, a maximum of 4 per cent of the substance is degraded in this manner.

Apart from a considerable quantity of psilocin excreted unaltered (25 per cent), the main metabolic reactions of the compound seem to be conjugations with as yet unidentified partners to form highly hydrophilic substances.

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