Biochemical Pharmacology, Vol. 18, pp. 2771–2781. Pergamon Press, 1969. Printed in Great Britain

METABOLISM OF 5-METHOXY-*N*,-*N* DIMETHYLTRYPTAMINE-^{14}C IN THE RAT

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(*Received* 9 *June* 1969; *accepted* 27 *June* 1969)

Abstract—The metabolism of 5-methoxy-*N*,*N*-dimethyltryptamine-^{14}C has been investigated in the rat after i.p. injection (5 mg/kg). About 59–65 per cent of the radioactivity is excreted in the 24 hr urine, 4–9 per cent is eliminated in the faeces and 1 per cent as carbon dioxide during this time. Of the metabolites appearing in the urine 54 per cent is present as 5-methoxyindoleacetic acid, 9 per cent as bufotenine, 23 per cent as bufotenine glucuronide and 14 per cent as 5-hydroxyindoleacetic acid. Considerable differences in the metabolism occurred at high dose levels. 5-Methoxyindoleacetic acid-^{3}H is *not* demethylated to 5-hydroxyindoleacetic acid. I.p. administered 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine-^{3}H is excreted mainly unchanged and to a minor degree as conjugate and as 6-hydroxy-5-methoxyindoleacetic acid.

5-Methoxy-*N*,*N*-dimethyltryptamine is not 6-hydroxylated to a detectable degree *in vivo* and 6-hydroxylation is apparently of no importance for the psychotropic activity of 5-methoxy-*N*,*N*-dimethyltryptamine.

5-METHOXY-*N*,*N*-DIMETHYLTRYPTAMINE (5-MeO-DMT) was isolated for the first time from plants (*Piptadenia colubrina*) in 1959 by Pachter *et al.*[1] This compound chemically closely related to serotonin also occurs in *Phalaris* species,[2] thereby causing the "stagger disease" in Australian sheep pastured on these perennial grasses. The potent effects of 5-MeO-DMT on the central nervous system was noted in 1962 by Gessner and Page[3] and 2 years later Holmstedt *et al.*[4] showed that 5-MeO-DMT was, in fact, the main component of certain psychoactive snuffs used by South American Indian tribes. The botanical aspects of South American plants yielding hallucinogenic snuffs have been described in detail[5] and an ethnopharmacologic description of the psychotomimetic effects of these drugs has been given by us.[6] Our recent investigations have shown 5-MeO-DMT to be a common constituent of South American hallucinogenic drugs and plants.[7,8]

In a previous paper,[9] where also the behavioral action and tremor-producing activity of 5-MeO-DMT was studied, we showed 5-methoxyindoleacetic acid to be the main metabolite of 5-MeO-DMT in the rat. The purpose of the present paper was to elucidate in detail the metabolic pathways of 5-MeO-DMT and particularly assess the importance of 6-hydroxylation as a metabolic pathway.

EXPERIMENTAL

Reference compounds

5-Methoxy-*N*,*N*-dimethyltryptamine-2′-^{14}C was synthetized as described earlier.[9] The specific activity was 0·93 mc/mM or 4·25 μc/mg and by radiochromatogram scanning, after paper chromatography with *n*-butanol–acetic acid–water (4:1:5) as solvent, it was shown to be 99·6 per cent pure containing about 0·2% of 5-methoxyindoleacetic acid dimethylamide. The latter compound was prepared from 5-methoxyindoleacetic acid and dimethylamine *via* the acid chloride.[10]

5-Methoxyindoleaceturic acid was synthetized from equimolar amounts of 5-methoxyindoleacetyl chloride and glycine methyl ester hydrochloride in ether-dimethyl formamide containing a large excess of triethylamine.[11] The compound was isolated as its methyl ester and the structure verified by mass spectrometry (M^+ 276) and infrared spectroscopy.

6-Hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine[12] (90 mg) was tritiated in 0·2 ml tritiated water (400 mc), 0·1 ml 1 M sulphuric acid and 0·1 ml water by heating in a sealed ampoule at 70° for 15 min. This labelling procedure is known to exchange only the hydrogens of the indole nucleus.[10,11] The product isolated after purification by TLC on Silica gel G using *n*-butanol–acetic acid–water (4:1:1) as solvent, had a specific activity of 370 μc/mM.

5-Methoxyindoleacetic acid-^{3}H was similarly prepared (100° for 3 hr) and recrystallized from ether, specific activity 1·79 mc/mM. The radiochemical purity of the tritium-labelled compounds was checked by TLC and GLC and was found to be over 99·5 per cent for 5-methoxyindoleacetic acid-^{3}H and over 95 per cent for the chemically unstable 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine-^{3}H. Other reference compounds have been described earlier.[9]

Animal experiments and excretion of labelled materials

5-MeO-DMT-^{14}C was given i.p. to 150–200 g male rats (Sprague–Dawley) in single doses of 5 or 10 mg/kg or, in repeated doses over 8 hr, 70 mg/kg. 5-Methoxyindoleacetic acid-^{3}H and 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine-^{3}H were injected i.p. in single doses of 10 mg/kg. The urine (urinary pH not controlled) and faeces were collected at different time intervals. Respiratory carbon dioxide was collected in 15% (v/v) ethanolamine in methanol. Radioactivity was determined by liquid scintillation counting (toluene–ethylene glycol monoethyl ether (1:1 v/v) containing 0·4% PPO and 0·01% dimethyl-POPOP). About 50 mg of dried faeces was dissolved in 1 ml of NCS solution (Nuclear Chicago Corp.) before adding the liquid scintillator.

Urine from male rats (450 g) injected with 6-hydroxy-5-methoxy-*N*,*N*-dimethoxyltryptamine-^{3}H, and in a few experiments also from 5-MeO-DMT-^{14}C, was collected directly, without exposure to air, in a continuously nitrogen-flushed vessel containing 1 ml 5% acetic acid. The movements of these experiment animals were restricted. Extraction of this urine was carried out immediately minimizing exposure to air.

Extraction of urine

Untreated and β-glucuronidase-treated urine from rats given 5-MeO-DMT-^{14}C and other labelled compounds was extracted as shown in Fig. 1. To obtain a better

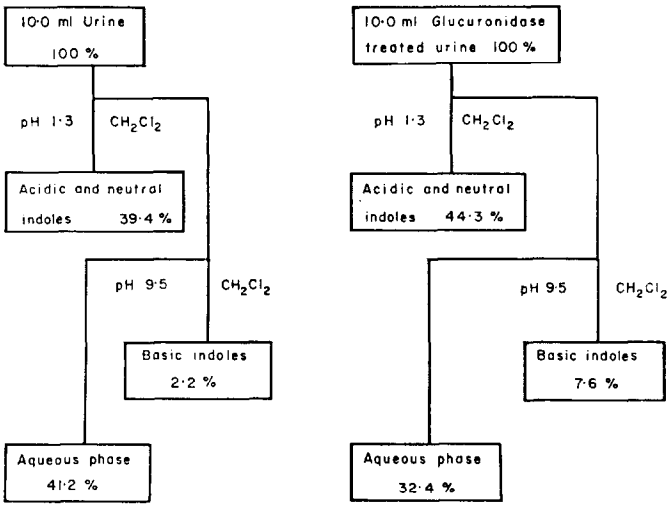

FIG. 1. Extraction scheme for untreated and glucuronidase-treated urine from rats given 5 mg/kg 5-MeO-DMT-^{14}C i.p. Percentage of radioactivity in each fraction indicated.

extraction of some possible metabolites of 5-MeO-DMT, e.g. serotonin, the alkaline aqueous phase was extracted with an equal volume of *n*-butanol.

Rat urine was adjusted to pH 7·0 and incubated once with bacterial β-glucuronidase (Type 1, Sigma Chemical Co.) in the presence of phosphate buffer pH 7·0 and chloroform at 37° overnight.

Separation of metabolites by gel filtration

Radioactivity in fractions from gel filtration columns was determined by liquid scintillation counting. Urea was estimated by the formation of a yellow colour with Ehrlich's reagent (3% *p*-dimethylaminobenzaldehyde in conc. HCl).

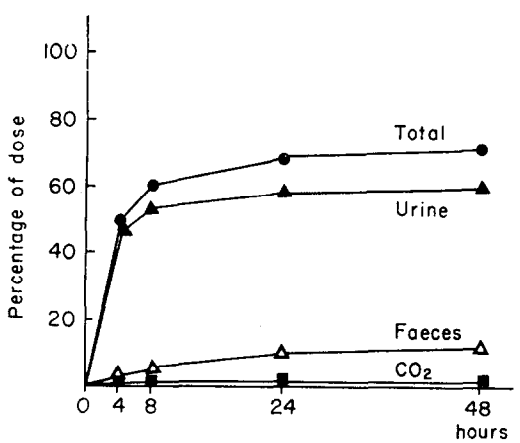

FIG. 2. Cumulative excretion of radioactivity in the urine, faeces and respiratory carbon dioxide after i.p. administration of 5-MeO-DMT-^{14}C (5 mg/kg). Average of four rats.

Gel filtration on Sephadex G-25. Thirty ml rat urine (24 hr collection) from animals given 5 mg/kg 5-MeO-DMT-^{14}C was lyophilised. Three ml of 50% aqueous ethanol was added and the filtered solution was transferred to a column of Sephadex G-25 Fine (1·5 × 180 cm). The column was eluted with 0·1% acetic acid at a rate of 9 ml/hr and fractions of 4·5 ml were collected (Fig. 3).

Gel filtration on Biogel P-2. Fractions 40–49 from the Sephadex column (Fig. 3),

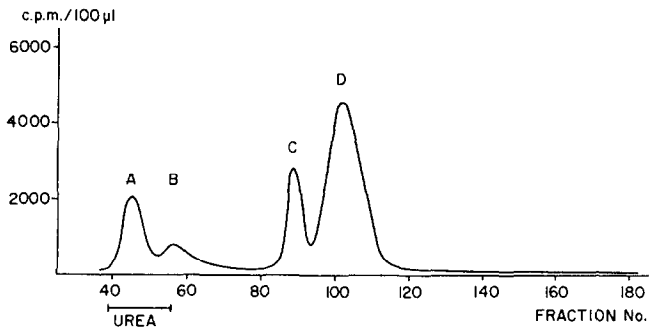

FIG. 3. Distribution of radioactivity after gel filtration on Sephadex G-25 of urine from rats given 5 mg/kg of 5-MeO-DMT-^{14}C.
A, mainly glucuronide of bufotenine; B, bufotenine; C, 5-hydroxyindoleacetic acid; D, 5-methoxyindoleacetic acid.

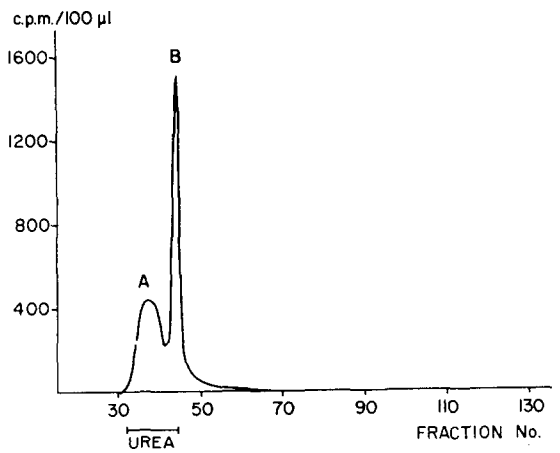

FIG. 4. Distribution of radioactivity in eluate from Biogel P-2 column. Fractions 40–49 from the Sephadex G-25 column rechromatographed on Biogel P-2. B, glucuronide of bufotenine.

concentrated to 2 ml, were rechromatographed on a column of Biogel P-2 (1·5 × 150 cm). Elution was carried out with 0·1% acetic acid at a rate of 13 ml/hr and fractions containing 6·5 ml were collected (Fig. 4).

Paper and thin-layer chromatography and radiochromatogram scanning

Paper chromatographic separation of metabolites of 5-MeO-DMT-^{14}C in urine

and comparison with references was carried out on Whatman 3 mm paper with *n*-butanol–acetic acid–water (4:1:5) or isopropanol–conc. ammonia–water (8:1:1) as solvents. Indoles were located with Ehrlich's reagent. Paper chromatograms were scanned in a Nuclear Chicago 4π Actigraph radiochromatogram scanner. The amount of each metabolite was calculated by planimetry. Separation of metabolites of 5-methoxyindoleacetic acid-^{3}H and 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine-^{3}H was carried out by TLC on Silica gel G with *n*-butanol–acetic acid–water (4:1:1) as solvent.

The distribution of activities of tritium was determined by counting scraped off sections of Silica gel G in liquid scintillator.

Gas chromatography and mass spectrometry

Gas chromatographic analyses were performed with an F & M model 400 apparatus with a flame ionization detector and a stream splitting device. Separations were carried out usually at 210° on 5% OV-17 on Gas Chrom P (100–120 mesh) in glass column (2·25 m × 3·2 mm i.d.). Acids were converted to methyl esters with diazomethane.[9]

Radioactivity in compounds eluted from the gas chromatograph was determined by use of a stream splitter and a Packard Model 850 Gas Fraction Collector. The mass spectrometric work was carried out with an LKB 9000 gas chromatograph-mass spectrometer as described previously.[9]

RESULTS

The excretion of radioactive material from 5-MeO-DMT-^{14}C (5 mg/kg, single dose) in urine, faeces and as respiratory carbon dioxide during 48 hr is shown in Fig. 2.

For the separation of radioactive metabolites from non-labelled compounds in the urine and from each other, two methods were used. One method consists of extractions with organic solvents at different pH (Fig. 1). Secondly, gel filtration was tested as a separation method. The presence and relative quantities of metabolites in different fractions was checked by paper chromatography–radiochromatogram scanning and by GLC.

Differential extraction of urine (5-MeO-DMT-^{14}C)

Urine, untreated or treated with β-glucuronidase (also containing sulphatase activity), was extracted as shown in Fig. 1. In this figure is also given the percentage of radioactivity in each fraction. A substantial amount of radioactivity is present in the extract containing neutral and acidic indoles and treatment with β-glucuronidase apparently slightly increases this amount. By a further separation into an acidic and neutral fraction, it was found that all the activity was present in two acidic indoles, later identified as 5-methoxyindoleacetic acid and 5-hydroxyindoleacetic acid. The most profound result of the β-glucuronidase treatment was the liberation of basic indoles and as expected a simultaneous decrease of radioactivity in the aqueous phase. The presence of bufotenine in the basic extracts was verified as shown below. At higher dose levels (70 mg/kg) also unchanged 5-MeO-DMT was present in the basic extracts (Fig. 5). As evident from the scheme a considerable amount of radioactive hydrophilic material remained in the aqueous phase after extraction. This activity was present mainly as bufotenine glucuronide.

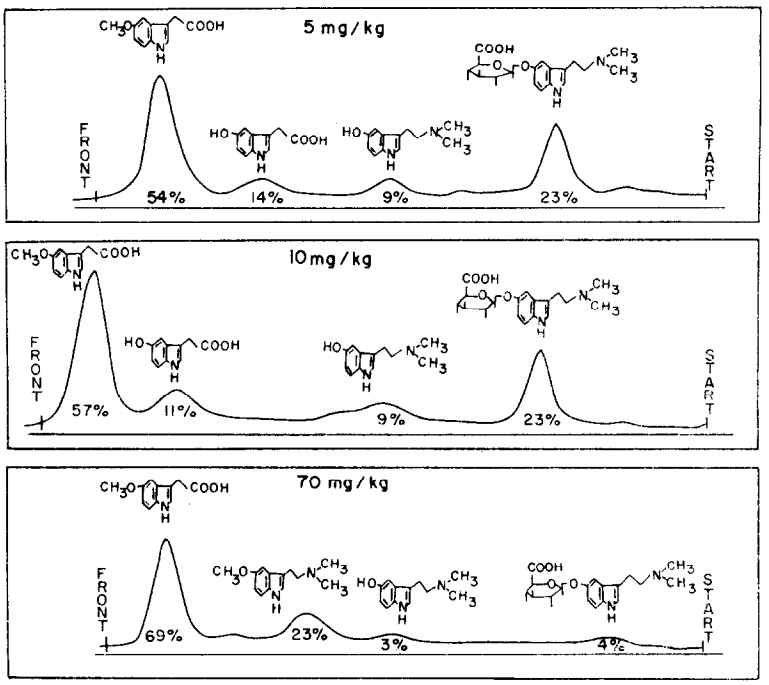

FIG. 5. Distribution of radioactivity in 24 hr urine from rats given 5 mg/kg (top), 10 mg/kg (middle) and 70 mg/kg (lower panel), of 5-MeO-DMT-^{14}C. Identity and percentage (determined by planimetry) of each metabolite shown. Top and lower panel, average of ten rats; middle panel, average of two rats. Paper chromatography on Whatman 3MM paper with *n*-butanol–acetic acid–water (4:1:5) as solvent.

Gel filtration of urine (5-MeO-DMT-^{14}C)

Gel filtration was also tested as a means of separating the different metabolites of 5-MeO-DMT-^{14}C from each other and from other urinary materials with the results shown in Fig. 3. Filtration of urine on Sephadex G-25 (Fig. 3) yielded particularly pure fractions of indolic acids which being aromatic were retained by the Sephadex matrix. The hydrophilic bufotenine glucuronide travels through Sephadex G-25 without any absorption effects and by rechromatography of fractions 40–49 from the Sephadex column on Biogel P-2 (Fig. 4) this compound was obtained free from other radioactive metabolites and almost free from urea.

Identification of metabolites of 5-MeO-DMT-^{14}C

5-Methoxyindoleacetic acid: Its identification as a metabolite of 5-MeO-DMT-^{14}C in the rat is based on the following criteria. The methyl ester of this compound was found by GLC to have a retention time identical with reference compound and the metabolite was further found to be radioactive. The metabolite was by comparison with authentic material identified by mass spectrum and by radiochromatogram scanning.

5-Hydroxyindoleacetic acid: This compound was identified as described above.

Bufotenine: This compound was identified by comparison with reference bufotenine as described for 5-methoxyindoleacetic acid.

Bufotenine glucuronide: After hydrolysis of the compound from Biogel P-2 (Fig. 4, peak B) with β-glucuronidase, a radioactive compound identical with bufotenine was obtained. Addition of 0·1 M phosphate as arylsulphatase inhibitor[13] had no effect on the liberation of bufotenine and thus it is concluded that the conjugate was a glucuronide. Indirect evidence rests upon the observation that upon treatment of the urine with β-glucuronidase the compound designated as bufotenine glucuronide (Fig. 5) largely disappears.

The following hypothetical metabolites of 5-MeO-DMT-^{14}C have been shown to be *absent* in urine from rats given 5 mg/kg 5-MeO-DMT-^{14}C. The maximum percentage in urine of a metabolite which may have been undetected is indicated:

5-methoxy-*N*,*N*-dimethyltryptamine	0·1%
5-methoxy-*N*-methyltryptamine	0·1%
5-methoxytryptamine	0·1%
5-hydroxytryptamine* (Serotonin)	1·0%
5-hydroxy-*N*-methyltryptamine*	1·0%
5-methoxytryptophol*	0·5%
5-hydroxytryptophol*	0·5%
5-methoxy-6-hydroxy-*N*,*N*-dimethyltryptamine*	0·1%
5-methoxyindoleaceturic acid	0·2%

* and as glucuronide.

Quantitative estimation of metabolites of 5-MeO-DMT-^{14}C

The metabolism of 5-MeO-DMT-^{14}C at dose levels of 5 mg/kg, 10 mg/kg (single doses) and 70 mg/kg (repeated doses under 8 hr) showed some differences as revealed by the urinary excretion (Fig. 5).

This experimental material was also used to calculate the amount of 5-MeO-DMT converted by competing biochemical reactions at different dose levels. These results are presented in Table 2.

Metabolism of 5-methoxyindoleacetic acid-^{3}H

Administration of this compound i.p. (10 mg/kg) resulted in a rapid elimination of

TABLE 1. GAS CHROMATOGRAPHIC DATA FOR REFERENCE INDOLES

Compound	Retention time (min) 210°	5% OV-17 247°
5-Methoxy-*N*,*N*-dimethyltryptamine	12·4	
5-Methoxytryptamine	13·1	
5-Methoxy-*N*-methyltryptamine	13·5	
5-Methoxytryptophol	14·1	
5-Hydroxy-*N*,*N*-dimethyltryptamine	17·7	
5-Hydroxytryptophol	20·4	
5-Methoxy-6-hydroxy-*N*,*N*-dimethyltryptamine	22·8	
5-Methoxyindoleacetic acid methyl ester	18·7	
5-Hydroxyindoleacetic acid methyl ester	27·7	6·0
5-Methoxy-6-hydroxyindoleacetic acid methyl ester	30·2	
5-Methoxyindoleaceturic acid methyl ester		30·5

radioactivity in the urine (68% in 4 hr; 77% in 8 hr; 83% in 24 hr—mean of three rats). Only 2% of the dose was eliminated in 24 hr via faeces.

In the 4 hr and the 8 hr urines over 99·5 per cent of the extracted labelled acid fraction consisted of the originally introduced acid. Incubation of the extracted aqueous phase with β-glucuronidase liberated some 5-hydroxyindoleacetic acid, which however was not radioactive. The urine collected between 8–24 hr contained minor amounts of 5-methoxy- and 5-hydroxyindoleacetic acids but likewise, only the methoxyindoleacetic acid was radioactive. The 5-hydroxyindoleacetic acid had in both cases a specific activity of less than 2 per cent of that of the methoxy acid isolated in the same extraction.

Metabolism of 6-*hydroxy*-5-*methoxy*-N,N-*dimethyltryptamine*-^3H

This compound when administered i.p. (10 mg/kg) was less rapidly eliminated with 36 per cent of the radioactivity (mean of three rats) excreted in the urine during 6 hr. Analysis by TLC of total urine and of acid and basic fractions (Fig. 1) as well as analysis by GLC, showed the presence in the urine of a main basic compound (31 per cent of dose) identified also by mass spectrometry, as unchanged 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine (Fig. 6 left part).

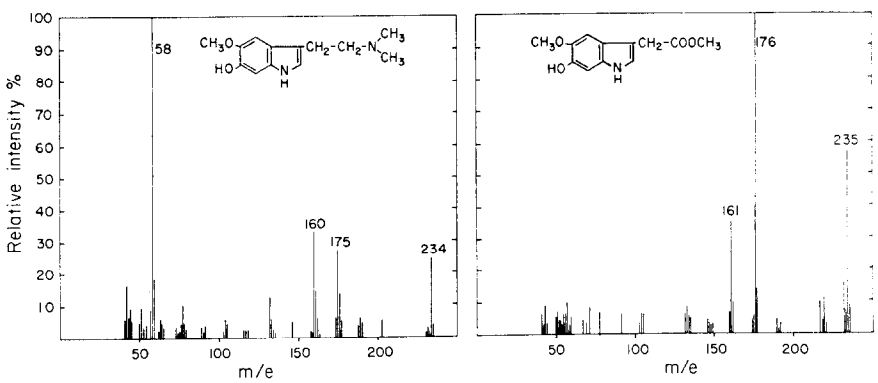

FIG. 6. Mass spectra of (left) 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine and (right) 6-hydroxy-5-methoxyindoleacetic acid methyl ester.

No other extractable basic metabolite could be detected. Only some 3 per cent of the dose was recovered in the acid fraction in 6 hr. Except for traces of the administered compound present also here, only one radioactive metabolite was present. The mass spectrum of the methyl ester of this acidic metabolite identified it as 6-hydroxy-5-methoxyindoleacetic acid (Fig. 6 right part).

A metabolite remaining in the extracted aqueous phase is apparently a conjugate (glucuronide or sulphate) of the introduced compound since incubation with β-glucuronidase yielded an additional 4 per cent of 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine. Other minor constituents undoubtedly are artefacts produced by air oxidation of the chemically unstable compounds[14] in spite of our precautions.

DISCUSSION

As mentioned in the introduction we have earlier shown[9] that 5-methoxyindoleacetic acid is the major metabolite of 5-MeO-DMT in the rat. Taborsky and McIsaac[15]

found, that 96 per cent of injected 5-methoxy-*N*-methyltryptamine was excreted in the urine as 5-methoxyindoleacetic acid, which also is the major metabolic product of 5-methoxytryptamine itself.[16] No other metabolites of 5-methoxytryptamine and its *N*-methyl derivatives are known.

As in our earlier study,[9] it was found (Fig. 2) that 5-MeO-DMT-^{14}C is rapidly eliminated in the urine with 59–65 per cent being excreted in 24 hr. Only 4–9 per cent is being eliminated during this time in faeces. There is little breakdown of the molecule since only 0·9 per cent of the injected 5-MeO-DMT-^{14}C is expired as radioactive respiratory carbon dioxide. Similar results have been obtained for psilocin-^{14}C, also labelled in the 2′-position, by Kalberer *et al.*[17] who recovered about 1 per cent of the administered radioactivity in the exhaled air.

Our present study shows, that 5-MeO-DMT-^{14}C in the rat at a dose level of 5–10 mg/kg i.p. is metabolized (Fig. 5) mainly to 5-methoxyindoleacetic acid (54–57 per cent of the metabolites in the urine). The remainder of the metabolites in the urine (Fig. 5) are the results of an initial *O*-demethylation of 5-MeO-DMT to bufotenine, which then partly undergoes further biochemical transformations to 5-hydroxyindoleacetic acid and bufotenine glucuronide. The amount of *O*-demethylated metabolites is considerable, i.e. 43–46 per cent of the metabolites excreted in the urine. 5-Hydroxyindoleacetic acid could possibly arise as a demethylation product of 5-methoxyindoleacetic acid but our further experiments with the latter compound showed that a detectable demethylation of 5-methoxy- to 5-hydroxyindoleacetic acid does not occur. This is evident from the fact that urine from rats injected i.p. with 5-methoxyindoleacetic acid-^{3}H contained beside the unchanged acid non-labelled 5-hydroxyindoleacetic acid showing that the 5-hydroxyindoleacetic acid was a metabolite of endogenous serotonin.

Thus, about half of the administered 5-MeO-DMT-^{14}C (5–10 mg/kg) is converted to bufotenine and there is a broad agreement with previous studies on the metabolism of bufotenine in the rat, cf. ref. 18, although minor quantitative differences exist. These may, however, be explained by experimental differences e.g. dose levels, extraction techniques, quantitation methods. Our present data (dose level 5–10 mg/kg) show that some 8–9 per cent of the urine metabolites is bufotenine, 11–14 per cent 5-hydroxyindoleacetic acid and 23 per cent bufotenine glucuronide. No other metabolite of 5-MeO-DMT was detected although the presence of a number of hypothetical metabolites was carefully investigated as discussed earlier under "*Identification of metabolites of 5-MeO-DMT-*^{14}*C*". No unchanged 5-MeO-DMT-^{14}C was detected at low dose levels and in agreement with Sanders and Bush[18] no sulphate conjugate of bufotenine could be detected. The metabolic pathways for 5-MeO-DMT in the rat are indicated in Fig. 7.

The metabolism of 5-MeO-DMT differed depending upon the dose given (Table 2, Fig. 5). The relative amounts of metabolites at dose levels of 5 and 10 mg/kg (single dose) were very similar. When the dose was increased to 70 mg/kg (several doses over 8 hr), which does cause pronounced toxic effects, there was a marked shift in the metabolic pathway. The figures given in Table 2 must be considered approximate, due to differences in administration and amount of radioactivity excreted in urine over 24 hr. However, it is clear that the total amount of *O*-demethylation and conjugation with glucuronate is similar at 10 mg/kg and 70 mg/kg, whereas the amount of metabolized substances by MAO to indolic acids is highly increased indicating that the

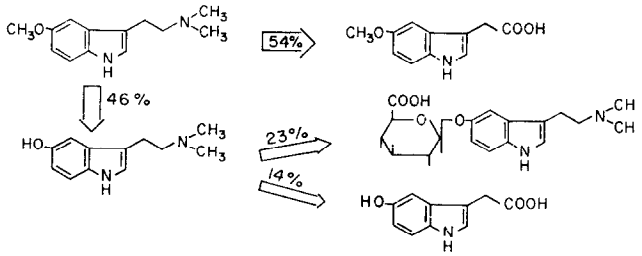

FIG. 7. Major pathways in the metabolism of 5-MeO-DMT in the rat (dose level 5 mg/kg).

TABLE 2. 5-MeO-DMT METABOLIZED TO VARIOUS METABOLITES AT DIFFERENT DOSE LEVELS, μM/kg*

5-MeO-DMT administered (mg/kg)	5	10	70
(μm/kg)	23	46	321
5-MeO-DMT appearing as metabolites in urine (% of administered dose in 24 hr urine) (μM/kg)	15(65%)	27(59%)	173(54%)
Metabolite formed (μM/kg)			
I. Bufotenine glucuronide	3·5	6·2	6·9
II. Bufotenine	1·3	2·4	4·3
III. 5-Hydroxyindoleacetic acid	2·1	3·0	1·7
IV. 5-Methoxyindoleacetic acid	8·1	15·4	119
Total *O*-demethylated metabolites (I-III)	6·9	11·6	12·9
Total metabolites oxidized to indole acids (III+IV)	10·2	18·4	120·7

*Approximate values calculated from the urinary output (24 hr urine) of metabolites (Fig. 5).

capacity of this enzyme is less limited than the enzymes carrying out *O*-demethylation and glucuronide conjugation. Some unchanged 5-MeO-DMT was excreted at 70 mg/kg.

Importance of 6-hydroxylation

Hydroxylation of indoles in the 6-position, as discussed at some length in our previous paper,[9] has been claimed to be an important metabolic route for indoles in man, monkey and rat.[19] The metabolites have been claimed to be psychoactive. Hence, the occurrence of 6-hydroxylated metabolites of 5-MeO-DMT was particularly investigated in the present study. In spite of considerable attempts no 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine could be detected in the urine.

The corresponding acid 6-hydroxy-5-methoxyindoleacetic acid is known to be very unstable in solution.[14] If any formed 6-hydroxylated 5-MeO-DMT was metabolized mainly to this acid in a similar way as 5-MeO-DMT is completely metabolized in low doses and mainly to the corresponding indolic acid, 6-hydroxylated metabolites of 5-MeO-DMT might have escaped our detection. We therefore decided to elucidate the previously not investigated metabolic fate of 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine-^{3}H administered i.p. (10 mg/kg) and collecting the urine under nitrogen without exposure to air. It was found, that 6-hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine is excreted mainly unchanged but also to some extent as conjugate and oxidized to 6-hydroxy-5-methoxyindoleacetic acid. 6-Hydroxylated basic derivatives

of similar amines e.g. α-methyltryptamine, *N*,*N*-diethyltryptamine and *N*,*N*-dimethyltryptamine are known to be eliminated in the urine, c.f. ref. 20.

Since urine from rats given 5-MeO-DMT was similarly collected under nitrogen without any 6-hydroxylated derivatives being detected (detection limit about 0·1 per cent of metabolites in urine) it can be safely stated, that the extent of 6-hydroxylation of 5-MeO-DMT in the normal rat is indeed minute and also for reasons discussed hereafter of no significance in the formation of psychoactive compounds.

6-Hydroxy-5-methoxy-*N*,*N*-dimethyltryptamine was compared with 5-MeO-DMT in their psychotropic effects on trained rats in a Skinner box and found to be less potent than the non-hydroxylated compound.[12] Similarly, Mitoma[21] using trained squirrel monkeys and the Wisconsin general test apparatus[22] found the non-hydroxylated 5-MeO-DMT to have much higher hallucinogenic properties. It could be argued that the 6-hydroxy compound entered the brain less readily. However, available data suggest that only microsomes from liver, not brain, can 6-hydroxylate indoles *in vitro*.[23]

Thus, 6-hydroxylation of 5-MeO-DMT is at most a minute metabolic path forming a less psychoactive metabolite and consequently of no importance for the psychoactive effects of the present compound.

Acknowledgements—This investigation was supported by Grant MH 12007 from The National Institute of Mental Health, U.S. Public Health Service, Chevy Chase, Md. USA, and in part (S.A.) by The Swedish Natural Science Research Council. Reference compounds were kindly provided by Drs. C. Mitoma, Stanford Research Institute, Menlo Park, and R. G. Taborsky, Cleveland Clinic Foundation, Cleveland, USA.

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