In Vivo Metabolism of 5-Methoxy-*N*,*N*-diisopropyltryptamine in Rat

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The *in vivo* metabolism of 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT), a psychoactive tryptamine analog, was studied in rat. Male Wistar rats were administered 10 mg/kg 5-MeO-DIPT hydrochloride orally, and urinary fractions were collected. After enzymatic hydrolysis, the metabolites were extracted by liquid-liquid extraction and analyzed by gas chromatography/mass spectrometry. 5-Methoxy-*N*-isopropyltryptamine, 5-hydroxy-*N*,*N*-diisopropyltryptamine (5-OH-DIPT), 5-hydroxy-*N*-isopropyltryptamine, and 5-methoxyindole-3-acetic acid were identified as 5-MeO-DIPT metabolites. By quantitative analysis using high-performance liquid chromatography, it was revealed that 5-OH-DIPT was the main metabolite of 5-MeO-DIPT in rat, comprising 20.5% of the dose administered. On the other hand, only 0.8% of 5-MeO-DIPT administered was excreted into the urine in its original form.

Key words —— 5-methoxy-*N*,*N*-diisopropyltryptamine, metabolism, rat, urine, quantitation

INTRODUCTION

5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT, Fig. 1) is a tryptamine analog with psychoactive properties. 5-MeO-DIPT was first reported by Shulgin and Carter in 1980.[1] According to their report, the subjective effects of 5-MeO-DIPT are substantially similar to those of 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and 4-bromo-2,5-dimethoxyphenethylamine (2C-B). The ingestion of 6–10 mg of 5-MeO-DIPT induces a psychoactive effect with an onset of about 20–30 min and a duration of about 3–6 hr. Subjects who have received 5-MeO-DIPT are talkative and disinhibited. At high doses, 5-MeO-DIPT produces nausea, jaw clenching, muscle tension, and overt hallucinations with both auditory and visual distortions.

In Japan, the abuse of 5-MeO-DIPT has become conspicuous in the past several years. A lot of web sites sell 5-MeO-DIPT, so abusers can purchase the drug easily. In recent years, the police have confiscated 5MeO-DIPT many times. In 2004, the Japanese Ministry of Health, Labour, and Welfare designated 5-MeO-DIPT as a narcotic under the Narcotics and Psychotropics Control Law.

To prove that a person has used a drug, it is necessary to identify the drug and its metabolites in biological samples such as urine and blood. The analytical data of 5-MeO-DIPT and its metabolites in biological samples have already been reported.[2–4] In those reports, 5-methoxy-*N*-isopropyltryptamine (5-MeO-IPT), 5-hydroxy-*N*,*N*-diisopropyltryptamine (5-OH-DIPT), 6-hydroxy-5-methoxy-*N*,*N*-diisopropyltryptamine (6-OH-5-MeO-DIPT), and 5-methoxyindole-3-acetic acid (5-MeO-IAA) were identified as metabolites of 5-MeO-DIPT in human urine.

In the present study, 5-MeO-DIPT hydrochloride was administered orally to rats and the urinary extracts were analyzed by GC/MS, then estimated urinary metabolites were confirmed by comparison with the authentic standards synthesized in our laboratory. Furthermore, the identified metabolites in urine were quantitatively analyzed by HPLC to clarify the metabolic behavior of 5-MeO-DIPT in rat.

MATERIALS AND METHODS

Materials —— 5-MeO-DIPT, *N*-isopropyltryptamine, and authentic standards of 5-MeO-DIPT metabolites except for 5-MeO-IAA were synthesized in our laboratory. The synthetic methods are described below. 5-MeO-IAA and 5-hydroxyindole-3-acetic acid (5-OH-IAA) were purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). β-Glucuronidase/aryl sulfatase (from *Helix pomatia*; β-glucuronidase, 6.76 units/ml; aryl sulfatase, 2.08 units/

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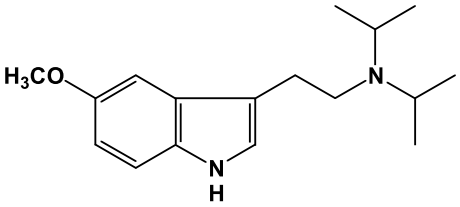

Fig. 1. Chemical Structure of 5-MeO-DIPT

ml) was purchased from Calbiochem-Novabiochem Co., Ltd. (La Jolla, CA, U.S.A.). All other chemicals used were of analytical grade.

Chemical Synthesis of 5-MeO-DIPT and its Metabolites —— All synthesized standards were confirmed by ^{1}H NMR. ^{1}H NMR spectra were measured with a JEOL JNM-ECP600 spectrometer. Tetramethylsilane was used as an internal standard.

5-MeO-DIPT: 5-MeO-DIPT hydrochloride was synthesized using procedures described by Shulgin.[5] ^{1}H-NMR (CD_3OD) δ1.42 (6H, d, J = 6.6 Hz), 1.44 (6H, d, J = 6.6 Hz), 3.19 (2H, m), 3.35 (2H, m), 3.80 (2H, m), 3.83 (3H, s), 6.80 (1H, dd, J = 2.5, 8.5 Hz), 7.03 (1H, d, J = 2.5 Hz), 7.21 (1H, s), 7.27 (1H, d, J = 8.5 Hz).

5-MeO-IPT: A 0.05 g sample of 5-methoxytryptamine hydrochloride was dissolved in 10 ml of water. The solution was made basic by adding 5 M sodium hydroxide solution and then was extracted with dichloromethane. After removing the solvent under vacuum, the residue was dissolved in 5 ml of ethanol with the addition of 0.2 ml of acetone and 0.05 g of palladium carbon (containing 5% palladium), followed by vigorous stirring under hydrogen gas for 24 hr. The catalyst was removed by filtration, and the solvent was evaporated to dryness under vacuum to give 0.054 g of 5-MeO-IPT. ^{1}H-NMR ($CDCl_3$) δ1.05 (6H, d, J = 6.6 Hz), 2.81 (1H, m), 2.94 (4H, s), 3.48 (3H, s), 6.86 (1H, dd, J = 2.2, 8.8 Hz), 7.02 (1H, d, J = 2.2 Hz), 7.07 (1H, d, J = 2.2 Hz), 7.25 (1H, d, J = 8.8 Hz).

5-OH-DIPT: To a suspension of 0.2 g of 5-benzyloxyindole-3-acetic acid in 5 ml of dichloromethane, 0.17 g of water-soluble carbodiimide hydrochloride (WSCI•HCl) and 0.12 ml of diisopropylamine were added, and the mixture was stirred for 1 hr. The reaction mixture was washed with 0.5 M acetate buffer (pH 5.0) and saturated sodium bicarbonate solution, then evaporated to dryness under vacuum. The residue was purified by silica-gel column chromatography to give 0.144 g of *N*,*N*-diisopropyl-5-benzyloxyindole-3-acetamide.

To a well-stirred solution of 0.144 g of *N*,*N*-diisopropyl-5-benzyloxyindole-3-acetamide in anhydrous tetrahydrofuran (THF) cooled on an ice bath, 0.75 g of lithium aluminum hydride was added, and the stirring continued for 15 min. The mixture was then refluxed for 40 hr. Once the reaction mixture was cooled to room temperature, water was carefully added until the vigorous reaction ceased. The reaction mixture was filtered, and the residual white solid was washed with methanol and THF. The filtrate was collected and THF was evaporated under vacuum. The remaining aqueous solution was made basic by the addition of 10 M sodium hydroxide solution and then extracted with dichloromethane. One half of the extract was purified by preparative thin layer chromatography (TLC), dissolved in 5 ml of ethanol, and then 0.01 g of palladium carbon (containing 5% palladium) was added, followed by vigorous stirring under hydrogen gas for 24 hr. The catalyst was removed by filtration, and the solvent was evaporated to dryness under vacuum to give 0.014 g of 5-OH-DIPT. ^{1}H-NMR ($CDCl_3$) δ1.14 (12H, d, J = 6.6 Hz), 2.74 (2H, m), 2.79 (2H, m), 3.16 (2H, m), 6.66 (1H, dd, J = 2.2, 8.8 Hz), 6.91 (1H, d, J = 2.2 Hz), 6.99 (1H, s), 7.15 (1H, d, J = 8.8 Hz).

5-Hydroxy-N-isopropyltryptamine (5-OH-IPT): A 0.05 g quantity of 5-OH-IPT was synthesized from 0.087 g of 5-benzyloxytryptamine and 0.2 ml of acetone by the same method used for the synthesis of 5-MeO-IPT. ^{1}H-NMR ($CDCl_3$) δ1.09 (6H, d, J = 6.1 Hz), 2.87 (1H, m), 2.92 (2H, t, J = 7.1 Hz), 2.99 (2H, t, J = 7.1 Hz), 6.79 (1H, dd, J = 2.2, 8.8 Hz), 6.96 (1H, d, J = 2.2 Hz), 6.99 (1H, s), 7.21 (1H, d, J = 8.8 Hz).

N-Isopropyltryptamine: A 0.076 g quantity of *N*-isopropyltryptamine was synthesized from 0.091 g of tryptamine and 0.2 ml of acetone by the same method used for the synthesis of 5-MeO-IPT. ^{1}H-NMR ($CDCl_3$) δ1.05 (6H, d, J = 6.1 Hz), 2.82 (1H, m), 2.94–3.01 (4H, m), 7.06 (1H, d, J = 2.2 Hz), 7.12 (1H, m), 7.20 (1H, m), 7.37 (1H, d, J = 7.7 Hz), 7.64 (1H, d, J = 7.7 Hz).

Animals and Treatments —— Four male Wistar rats were orally administered 10 mg/kg of 5-MeO-DIPT hydrochloride and placed in metabolic cages. The 0–24 and 24–48 hr urinary fractions were collected and stored at –20°C until used for analysis. All experiments were approved by the Animal Ethics Committee of National Research Institute of Police Science.

Identification of the Metabolites —— After 5 ml of each urine sample was adjusted to pH 5 with ace-

tic acid, 1.25 ml of 0.5 M acetate buffer (pH 5.0) and β-glucuronidase/aryl sulfatase (8 μl of enzyme/ml urine) were added to the urine and incubated for 2.5 hr at 60°C. Hydrolyzed urine was adjusted to pH 2 with 3 M hydrochloric acid and then extracted with diethylether. The organic layer was back-extracted with 0.5 M sodium hydroxide solution. The organic layer was defined as fraction 1. The aqueous layer was acidified to pH 2 with 3 M hydrochloric acid and again extracted with diethylether. This organic layer was defined as fraction 2. In addition, the remaining aqueous layer from the first extraction step was taken to pH 12 with a 5 M sodium hydroxide solution and extracted with chloroform. This organic layer was defined as fraction 3. The aqueous layer was neutralized by adding 3 M hydrochloric acid and the pH was then adjusted to about 9 by the addition of a 25% ammonium hydroxide solution and extracted with chloroform/2-propanol (3 : 1). The organic layer was defined as fraction 4. Each fraction was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The residues of fraction 1, 3, and 4 were reconstituted in 1 ml of methanol for GC-MS analysis. The residue of fraction 2 was reconstituted in 1 ml of methanol. A 50 μl portion of the solution was transferred to another vial, and then the solvent was evaporated to dryness under a gentle stream of nitrogen. The residue, dissolved in 50 μl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), was heated at 80°C for 30 min for trimethylsilyl (TMS) derivatization, and was then analyzed by GC/MS.

Quantitation of the Metabolites —— To a 0.2 ml of urine, 0.8 ml of 0.1 M acetate buffer (pH 5.0) containing β-glucuronidase/aryl sulfatase (10 μl of enzyme/ml) was added and heated at 60°C for 2.5 hr for enzymatic hydrolysis. One-half milliliter of 0.5 M borate buffer (pH 9.0) was added to the hydrolysis sample, which was then extracted three times with chloroform/2-propanol (9 : 1) after adjusting the pH to 9 with 10% sodium carbonate solution (basic fraction). The remaining aqueous layer was acidified to pH 2 with 3 M hydrochloric acid and extracted with chloroform/2-propanol (9 : 1) (acidic fraction). For the quantitation of unconjugated forms, in the basic fraction, the step involving β-glucuronidase/aryl sulfatase hydrolysis was omitted (water was added to the urine instead of acetate buffer containing enzyme). The basic fraction was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum after adding 10 μg of *N*-isopropyltryptamine as an internal standard, reconstituted in 200 μl of mobile phase, then analyzed by HPLC. The acidic fraction was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum after adding 10 μg of 5-benzyloxyindole as an internal standard. The residue, dissolved in 50 μl of MSTFA, was heated at 80°C for 30 min for TMS derivatization, and was then analyzed by GC/MS.

GC/MS —— GC/MS analysis was performed using a Finnigan•MAT GCQ (San Jose, CA, U.S.A.) equipped with a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, U.S.A.). The oven temperature was held at 80°C for 1 min following injection and then programmed to 280°C at a rate of 15°C/min. The injection port and interface temperature were set at 200 and 275°C, respectively. Helium was used as carrier gas at a flow rate of 1 ml/min. The mass spectrometer was operated under the electron ionization (EI) mode at an ionization energy of 70 eV. Samples were injected in the splitless mode.

HPLC —— HPLC analysis was performed using an HPLC system consisting of an LC-10AD*VP* liquid chromatograph, an SIL-10AD*VP* auto injector, a CTO-10AC*VP* column oven, and an SPD-M10A*VP* diode array detector (Shimadzu, Kyoto, Japan) equipped with a CAPCELL PAK SCX column (250 × 1.5 mm i.d., Shiseido, Tokyo, Japan). The mobile phase consisted of 30 mM ammonium acetate (pH 5.0)/acetonitrile (40 : 60), and the flow rate was 0.15 ml/min. The oven temperature was held at 40°C.

Calibration Curve —— Authentic standards of 5-MeO-DIPT and its known metabolites were added to the urinary samples, and were processed as described in the section of quantitation of the metabolites, and then were analyzed by HPLC to obtain the calibration curves. Excellent linearity was obtained over the concentration range 1.2–60 μg/ml with a correlation coefficient of 0.999.

RESULTS AND DISCUSSION

To identify the metabolites of 5-MeO-DIPT in rat urine, the four fractions obtained from the urine by liquid-liquid extraction were analyzed by GC/MS.

Figure 2 shows the total ion chromatogram (TIC) and mass chromatograms obtained from the TMS derivative of fraction 2. The acidic compounds are included in this fraction. The mass spectra of peak A and peak B are shown in Figure 3. By a comparison of the retention times and mass spectra of these

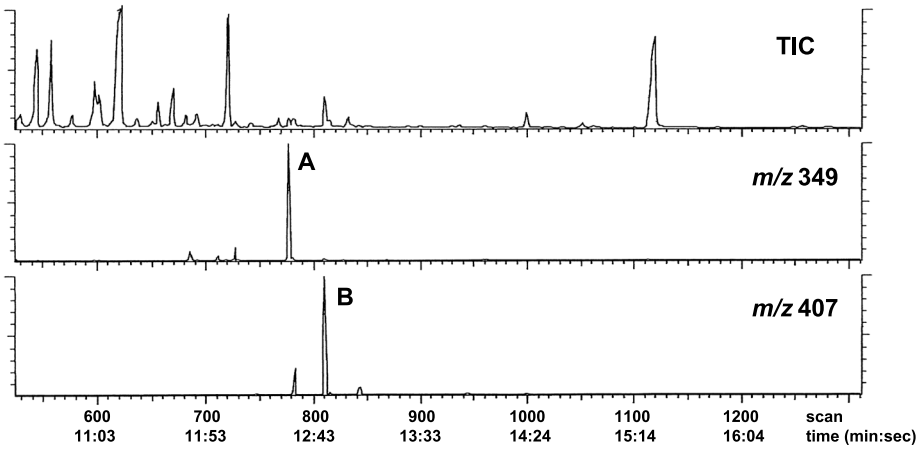

Fig. 2. Total Ion Chromatogram (TIC) and Mass Chromatograms under the EI Mode Obtained from TMS Derivative of Fraction 2

peaks with those of the authentic standards, peaks A and B were identified as 5-MeO-IAA and 5-OH-IAA, respectively.

Figure 4 shows the TIC obtained from fraction 3. The basic compounds are included in this fraction. The mass spectra of peaks C–F are shown in Fig. 5. Peak E was identified as unchanged 5-MeO-DIPT. Peak C was presumed to be the 5-MeO-IPT, because the ion at *m/z* 232 was detected as a molecular ion and the ion at *m/z* 161 is formed by the cleavage at the α-bond. Peak D was presumed to be 5-OH-IPT, because the molecular ion (*m/z* 218) and α-cleavage product (*m/z* 147) were detected, as they were with 5-MeO-IPT. Peak F was presumed to be 5-OH-DIPT, because the ions containing the indole ring at *m/z* 160 and 146 are formed by the cleavage at the C-N bond and α-bond, respectively, whereas ions at *m/z* 114 and 72 were observed as they were with 5-MeO-DIPT. These were confirmed by a comparison of the retention time and the mass spectrum of authentic standards synthesized in our laboratory. No metabolites were detected in fraction 1 and 4.

For the quantitation of the metabolites, urine samples were processed by liquid-liquid extraction using chloroform/2-propanol (9 : 1) under the weak alkaline condition, followed by extraction under the strongly acidic condition, after which the alkaline and acidic fractions were analyzed by HPLC and GC/MS after TMS derivatization, respectively. The recovery of each metabolite is summarized in Table 1. While the recovery of 5-OH-IPT was slightly low (79.5%), the other metabolites and unchanged 5-MeO-DIPT were recovered at satisfactory rates ranging from 92.2 to 99.8%.

The excretory profile of 5-MeO-DIPT in rat urine

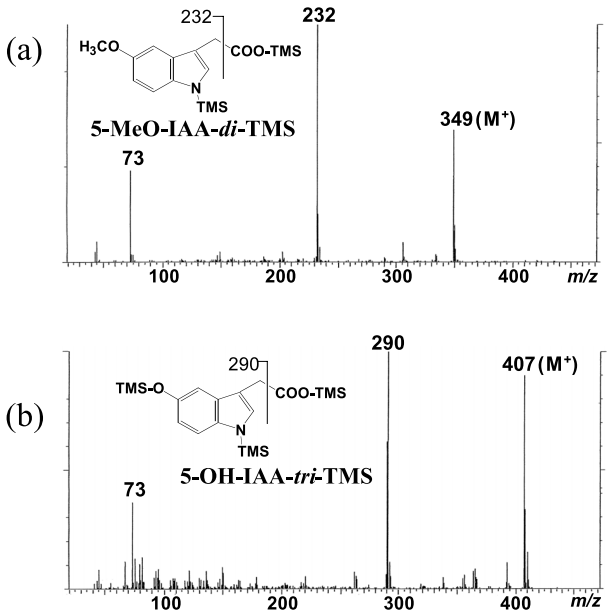

Fig. 3. Mass Spectra of Peak A (a) and Peak B (b)

is summarized in Table 2. The main metabolite detected in urine was 5-OH-DIPT, accounting for 20.5% of the dose in 24 hr. The recovery of the free form of this metabolite was only 1.8% of the dose, indicating that almost all of this metabolite was excreted as the conjugated form. The amounts of the other metabolites were, in descending order, 5-OH-IPT (3.6%), 5-MeO-IAA (3.4%), and 5-MeO-IPT (2.6%). The percentage of unchanged 5-MeO-DIPT excreted in urine was as low as 0.8%. In the 24–48 hr urinary fractions, only 5-OH-DIPT was detected with very small amount (0.4%), indicated that 5-MeO-DIPT and its metabolites were rapidly eliminated from the rat body. 5-OH-IAA was thought to

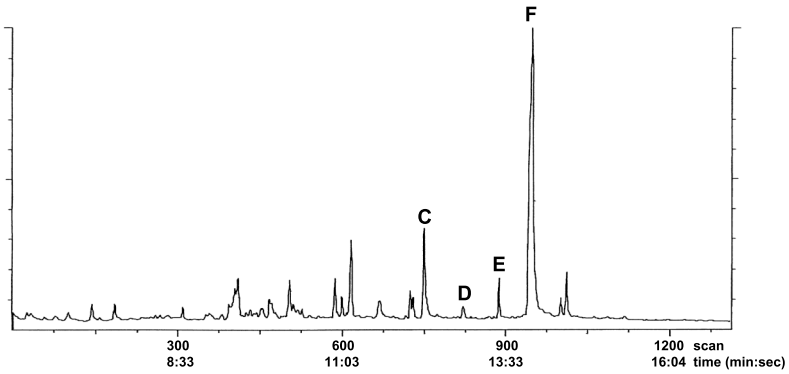

Fig. 4. TIC under the EI Mode Obtained from Fraction 3

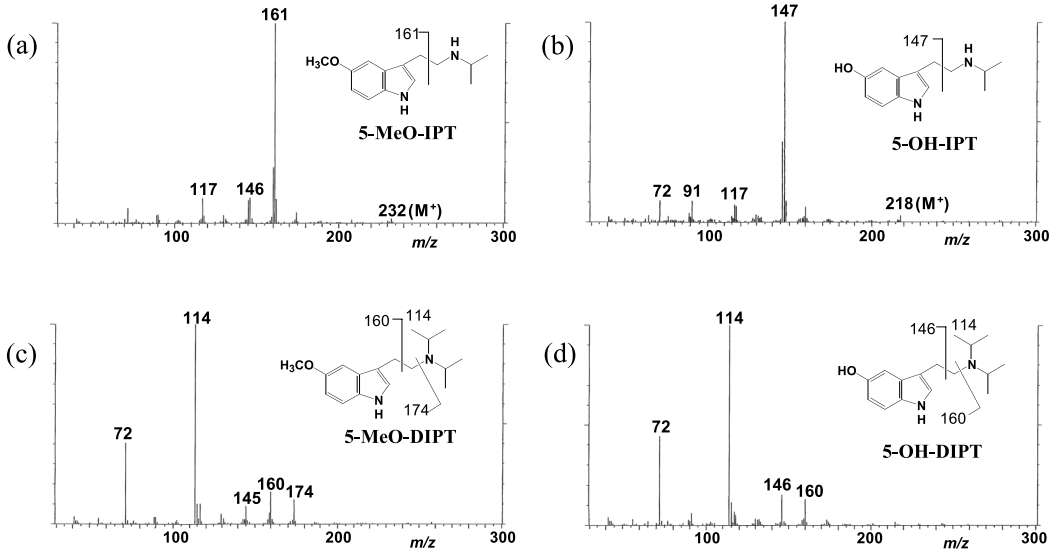

Fig. 5. Mass Spectra of Peak C (a), Peak D (b), Peak E (c) and Peak F (d)

Table 1. Recovery of 5-MeO-DIPT and its Metabolites from Spiked Urine Sample

Compound	Recovery (%)	
	Acidic fraction	Basic fraction
5-MeO-DIPT	—	98.9 ± 2.0
5-MeO-IPT	—	99.8 ± 2.6
5-OH-DIPT	—	92.2 ± 2.1
5-OH-IPT	—	79.5 ± 3.5
5-MeO-IAA	95.9 ± 4.0	—

Blank urine samples were spiked with each compound at a concentration of 50 μg/ml. Data represent the mean ± S.D. of four determinations.

be yielded from 5-OH-IPT or 5-MeO-IAA by oxidative deamination or *O*-desmethylation,[3] respectively. On the other hand, 5-OH-IAA was also an endogenous serotonin metabolite.[6] The level of 5-OH-IAA in 5-MeO-DIPT-administered rat urine was somewhat higher than control rat urine according to the semi-quantitative measurement (data not shown), indicating that 5-OH-IAA was formed from 5-MeO-DIPT in rat. However, 5-OH-IAA was not a major metabolite of 5-MeO-DIPT, since the amount of 5-OH-IAA derived from 5-MeO-DIPT was thought to be less than 1% of the administered dose (data not shown).

The proposed metabolic pathway of 5-MeO-DIPT in rat is shown in Fig. 6. 5-MeO-DIPT is metabolized by *O*-desmethylation, *N*-desisopropylation, and oxidative deamination. Recently, Narimatsu *et al*. reported that 5-MeO-DIPT is *O*-desmethylated by CYP2D6 and is *N*-desisopropylated by CYP1A2, CYP2C8 and CYP3A4 in the *in vitro* experiment using human liver microsomes and recombinant cytochrome P450 enzymes.[7] Cytochrome P450 enzymes may also catalyze the *O*-desmethylation and

Table 2. Excretion of 5-MeO-DIPT and its Metabolites in the Urine of Rat

Compound	% of the primed dose of 5-MeO-DIPT			
	0–24 hr fraction		24–48 hr fraction	
	Total	Free	Total	Free
5-MeO-DIPT	0.8 ± 0.2	0.8 ± 0.2	ND	ND
5-MeO-IPT	2.6 ± 0.2	2.6 ± 0.2	ND	ND
5-OH-DIPT	20.5 ± 2.4	1.8 ± 0.3	0.4 ± 0.3	ND
5-OH-IPT	3.6 ± 0.8	0.2 ± 0.1	ND	ND
5-MeO-IAA	3.4 ± 0.4	3.4 ± 0.4	ND	ND

Data represent the mean ± S.D. of four rats.

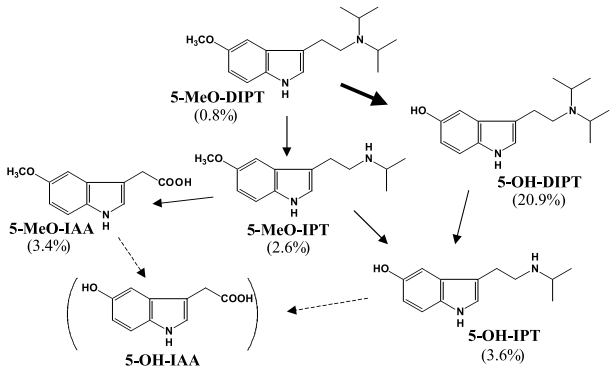

Fig. 6. Proposed Metabolic Pathway for 5-MeO-DIPT in Rat

the *N*-desisopropylation of 5-MeO-DIPT in rat. 5-OH-DIPT is the most abundant metabolite in urine, so *O*-desmethylation is thought to be the major metabolic pathway of 5-MeO-DIPT in rat. However, the total amount of metabolites identified in urine is only about 30%, and the remaining 70% is still unknown. The remaining metabolites may be excreted into faeces, or they may not be recovered by liquid-liquid extraction due to their strong hydrophilicities. 5-OH-IPT is a new metabolite of 5-MeO-DIPT first reported in this study. According to the previous reports,[2–4] both of *O*-desmethylated metabolite 5-OH-DIPT and *N*-desisopropylated metabolite 5-MeO-IPT were identified in human urine as in case with rat, so the formation of 5-OH-IPT, which was formed by both of *O*-desmethylation and *N*-desisopropylation, may be possible in human. 5-OH-IPT have never been detected in human urine, it may be due to the low concentration of this metabolite in human urine. The data we have presented in this study will very useful for the analysis of 5-MeO-DIPT and its metabolites in forensic samples.

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