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METABOLISM OF THE PSYCHOTOMIMETIC TRYPTAMINE DERIVATIVE 5-METHOXY-*N,N*-DIISOPROPYLTRYPTAMINE (5-MEO-DIPT) IN HUMANS

Identification and Quantification of its Urinary Metabolites

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Abbreviations

CID	collision-induced dissociation
DMT	dimethyltryptamine
EI	electron ionization
ESI	electrospray ionization
GC	gas chromatography
5-HIAA	5-hydroxyindoleacetic acid
I.S.	internal standard

LC	liquid chromatography
5-MeO-DMT	5-methoxty-*N,N*-dimethyltryptamine
5-MeO-DIPT	5-methoxy-*N,N*-diisopropyltryptamine
5-MeO-DIPT-NO	5-methoxy-*N,N*-diisopropyltryptamine-*N*-oxide
5-MeO-NIPT	5-methoxy-*N*-isopropyltryptamine
MS	mass spectrometry
MSTFA	*N*-methyl-*N*-trimethylsilyltrifluoroacetamide
5-MT	5-methyltryptamine
5-OH-DIPT	5-hydroxy-*N,N*-diisopropyltryptamine
6-OH-5-MeO-DIPT	6-hydroxy-5-methoxy-*N,N*-diisopropyltryptamine
SIM	selected ion monitoring
THF	tetrahydrofuran
TMS	trimethylsilyl

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ABSTRACT:

The urinary metabolites of 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) in humans have been investigated by analyzing urine specimens from its users. For the unequivocal identification and accurate quantification of its major metabolites, careful analyses were conducted by gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry, and liquid chromatography/mass spectrometry/mass spectrometry, using authentic standards of each metabolite synthesized. Three major metabolic pathways were revealed as follows: 1) side-chain degradation by *O*-demethylation to form 5-hydroxy-*N,N*-diisopropyltryptamine (5-OH-DIPT) which would be partly conjugated to its sulfate and glucuronide; 2) direct hydroxylation on position 6 of the aromatic ring of 5-MeO-DIPT, and/or methylation of the hydroxyl group on position 5 following hydroxylation on position 6 of the aromatic ring of 5-OH-DIPT, to produce 6-hydroxy-5-methoxy-*N,N*-diisopropyltryptamine (6-OH-5-MeO-DIPT), followed by conjugation to its sulfate and glucuronide; and 3) side-chain degradation by *N*-deisopropylation, to the corresponding secondary amine 5-methoxy-*N*-isopropyltryptamine (5-MeO-NIPT). Of these metabolites which retain structural characteristics of the parent drug, 5-OH-DIPT and 6-OH-5-MeO-DIPT were found to be more abundant than 5-MeO-NIPT. Although the parent drug 5-MeO-DIPT was detectable even 35 h after dosing, no trace of its *N*-oxide was detected in any of the specimens examined.

Introduction

5-Methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) is a tryptamine derivative which possesses the critical features in its chemical structure necessary for hallucinogen/stimulant activity; substitutions on the indole ring and the ethylamine side-chain of tryptamine result in a pharmacologically active substance (Fig. 1). Shulgin and Carter (1980) synthesized 5-MeO-DIPT and reported its hallucinogenic activity when administered orally. It produces pharmacological effects similar to those of other Schedule I hallucinogens such as dimethyltryptamine (DMT) (Shulgin and Carter, 1980). It is often used orally at a dosage ranging from 6 to 20 mg, and is sometimes taken through the routes of smoking and snorting. Oral administration of 6-10 mg of 5-MeO-DIPT produces subjective effects with an onset at about 20-30 min, a peak at about 1-1.5 h, and a duration of about 3-6 h (Drug Enforcement Administration (DEA), Department of Justice, 2003). Also, 5-MeO-DIPT causes mydriasis, and high dosages produce nausea, jaw clenching, muscle tension, and overt hallucinations with both auditory and visual distortions.

The abuse problem of 5-MeO-DIPT first emerged in 2001 in the United States (Drug Enforcement Administration (DEA), Department of Justice, 2001), and in the same year in Japan (Katagi et al., 2002) and since then, in various other countries. 5-MeO-DIPT has often been sold under the name "Foxy" or "Foxy Methoxy" in tablet and capsule forms, which is often used in raves (lively parties involving dancing and drinking). Since this drug is easily available through the Internet, its use has been extensively and rapidly spreading, especially among teenagers and young adults. Because of its escalating popularity and potent physiological effects, increasing numbers of acute poisoning cases (e.g., its overdose) have been reported. Thus 5-MeO-DIPT was amended into Schedule I of the Controlled Substances Act in 2003

in the United States (Drug Enforcement Administration (DEA), Department of Justice, 2003), and was banned in Japan in April, 2005.

Although research on the metabolism of 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) in mammals (Sitaram et al., 1987) provides some useful information for analyzing 5-MeO-DIPT metabolites in human body fluids, only a few data have been reported for its metabolism in humans. Recently, on metabolites in humans, "tentative identification" of several metabolites but without using authentic standards were reported (Meatherall and Sharma, 2003; Wilson et al., 2005).

In this report we have aimed for the careful investigation and indisputable identification of the metabolites of 5-MeO-DIPT in humans. The authors for the first time synthesized the authentic standards of its metabolites that were predicted based on previous studies (Meatherall and Sharma, 2003; Wilson et al., 2005; Tsutsumi et al., 2005a; Tsutsumi et al., 2005b). Utilizing the authentic standards, several urine specimens from 5-MeO-DIPT users were analyzed by gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), and liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). The excretion profiles are presented, and the metabolic pathways of 5-MeO-DIPT are discussed.

Experimental

Materials.

5-MeO-DIPT hydrochloride, 5-methoxy-*N*-isopropyltryptamine (5-MeO-NIPT) hydrochloride, 5-hydroxy-*N,N*-diisopropyltryptamine (5-OH-DIPT) hydrochloride, 6-hydroxy-5-methoxy-*N,N*-diisopropyltryptamine (6-OH-5-MeO-DIPT), and 5-methoxy-*N,N*-diisopropyltryptamine-*N*-oxide (5-MeO-DIPT-NO) were synthesized in our laboratory according to previously published methods with partial modification, as detailed in the subsequent section (Their chemical structures can be seen in Fig. 5). Every synthesized compound was ensured to be >95% pure based on high-resolution MS analysis by the flow-injection method. Stock standard solutions of these five compounds were prepared in methanol (1 mg/ml each), and then diluted to appropriate concentrations with distilled water or control human urine, immediately prior to use. The internal standard (I.S.) 5-methyltryptamine (5-MT) hydrochloride was purchased from Aldrich (Milwaukee, WI, USA), and an I.S. solution (200 ng/ml) was prepared in distilled water. Acetonitrile and methanol were of HPLC-grade, and other chemicals used were of analytical grade. Sulfatase/β-glucuronidase (*Helix pomatia*, Type H-1) was obtained from Sigma (St. Louis, MO, USA). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) used for trimethylsilyl (TMS) derivatization was purchased from Wako (Osaka, Japan).

Chemical Synthesis.

5-MeO-DIPT (I) ***I*** was synthesized from 5-methoxytryptamine and purified, according to the method of Shulgin and Shulgin (Shulgin and Shulgin, 1997a).

5-MeO-NIPT (II) ***II*** was synthesized according to the above-mentioned procedure, with a slight modification: Instead of excess 2-iodopropane, an equivalent

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amount of 2-iodopropane was added to 5-methoxytryptamine (Aldrich), and the resultant reaction mixture was purified on a column packed with Chromatorex NH, using chloroform as an eluent (Fuji Silysia Chemical, Ltd., Kasugai, Japan).

5-OH-DIPT (III) ***III*** was synthesized in analogy to the modified procedure for 5-hydroxy-*N,N*-dimethyltryptamine (Shulgin and Shulgin, 1997b): Diisopropylamine was used instead of dimethylamine.

6-OH-5-MeO-DIPT (IV) ***IV*** was synthesized according to the procedures of Shulgin and Shulgin (1997c; 1997d), with modifications as follows: To concentrated nitric acid which was stirred and cooled in an external ice-bath, finely powdered 4-benzyloxy-3-methoxybenzaldehyde (Avocado Research Chemicals, Ltd., Heysham, UK) was added. (The temperature must not be allowed to rise above 0°C.) After 2 h of additional stirring, the reaction mixture was poured over chipped ice, and the product was extracted with ethylacetate. The extract was washed with a saturated aqueous sodium bicarbonate solution and water to remove remaining acid. After evaporation, the residue was subjected to column chromatography, using a silica gel column and an ethylacetate-*n*-hexane mixture (1:1, v/v) as a developing solvent, to obtain 2-nitro-4-benzyloxy-3-methoxybenzaldehyde (The yield in this step was 70%).

A solution of 2-nitro-4-benzyloxy-3-methoxybenzaldehyde in glacial acetic acid was treated with nitromethane (Wako) followed by with anhydrous ammonium acetate. After being held at reflux for 2 h, the reaction mixture was poured into ice water. The product was extracted with ethylacetate, and the extract was evaporated to dryness after washing with water. The residue was subjected to column chromatography, using a silica gel column and an ethylacetate-*n*-hexane mixture (1:2, v/v) as a developing solvent, to isolate 2,2'-dinitro-4-benzyloxy-3-methoxystyrene (78%).

To glacial acetic acid, 2,2'-dinitro-4-benzyloxy-3-methoxystyrene and electrolytic powdered iron was added, stirred, and heated gently until an exothermic reaction set in, and this was maintained at a controlled pace with external cooling. When the spontaneous reaction had subsided, the reaction mixture was refluxed for 15 min, cooled, neutralized with sodium hydroxide, extracted with ethylacetate. The extract was evaporated. The residue was subjected to column chromatography, using a Chromatorex NH column and an ethylacetate-*n*-hexane mixture (1:3, v/v) as a developing solvent, to obtain 6-benzyloxy-5-methoxyindole (30%).

To a well-stirred, cold solution of 6-benzyloxy-5-methoxyindole in anhydrous diethylether, a solution of oxalyl chloride (Wako) in diethylether was added dropwise with protection from atmospheric moisture. The reaction mixture was stirred for an additional 20 min, and the intermediate indoleglyoxyl chloride separated as a crystalline solid but was not isolated. This was treated with 40% solution of diisopropyl amine in anhydrous diethylether, dropwise, until the pH reached 8-9. The reaction mixture was diluted with chloroform and shaken with 5% aqueous potassium bisulfate (Wako) solution, followed by with a saturated aqueous sodium bicarbonate solution. After drying, the organic solvent was removed. The residue was subjected to column chromatography, using a Chromatorex NH column and an ethylacetate-*n*-hexane mixture (1:5, v/v) as a developing solvent, to isolate 6-benzyloxy-5-methoxyindol-3-yl-*N,N*-diisopropylglyoxylamide (55% in these two steps).

To a well-stirred suspension of lithium aluminum hydride in dry tetrahydrofuran (THF), a solution of 6-benzyloxy-5-methoxyindol-3-yl-*N,N*-diisopropylglyoxylamide in anhydrous THF was added dropwise. The mixture was brought to a reflux temperature, held there for 15 min, and allowed to return to room temperature. The

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excess hydride was destroyed by adding ethylacetate and water. The solids were removed by filtration, the filter cake washed with THF, and the pooled filtrate and washings were evaporated under vacuum. The residue was subjected to column chromatography, using a Chromatorex NH column and an ethylacetate-*n*-hexane mixture (1:5, v/v) as a developing solvent, to obtain 6-benzyloxy-5-methoxy-*N,N*-diisopropyltryptamine (35%).

A solution of 6-benzyloxy-5-methoxy-*N,N*-diisopropyltryptamine in methanol containing 10% palladium-activated carbon catalyst was shaken under 3 atm hydrogen for 6 h, and the solids were removed by filtration. Evaporation of the solvent under vacuum gave a residue. The residue was subjected to column chromatography using a Chromatorex NH column and an ethylacetate-*n*-hexane mixture (1:1, v/v) as a developing solvent, to isolate 6-hydroxy-5-methoxy-*N,N*-diisopropyltryptamine (88%). The overall isolated yield was 2.8%.

5-MeO-DIPT-NO (V) ***V*** was synthesized from ***I***, according to the procedure of Cymerman Craig and Purushothaman (Cymerman Craig and Purushothaman, 1970).

The identities of all synthesized compounds were confirmed by high-resolution MS and MS/MS in the flow-injection method, in addition to GC/EI MS with and without TMS derivatization and LC/ESI MS. The GC/EI MS and LC/ESI MS data are described in the Results section. The high-resolution mass spectral data (calculated exact mass is given in the parentheses) are as follows: ***I***: *m/z* 275.2102 $[M+H]^+$ (275.2133), 174.0896 $[M-(C_3H_7)_2N]^+$ (174.0919), 114.1314 $(C_3H_7)_2NCH_2^+$ (114.1283). ***II***: *m/z* 233.1659 $[M+H]^+$ (233.1654), 174.0894 $[M-C_3H_7NH]^+$ (174.0919). ***III***: *m/z* 261.1967 $[M+H]^+$ (261.1967), 160.0756 $[M-(C_3H_7)_2N]^+$ (160.0762), 114.1312

$(C_3H_7)_2NCH_2^+$ (114.1283). ***IV***: *m/z* 291.2079 $[M+H]^+$ (291.2072), 190.0855 $[M-(C_3H_7)_2N]^+$ (190.0852). ***V***: *m/z* 291.2099 $[M+H]^+$ (291.2072), 174.0897 $[M-(C_3H_7)_2NO]^+$ (174.0919).

Apparatus.

GC/MS was carried out on a GCMS QP-2010 (Shimadzu, Kyoto, Japan). A fused-silica capillary column DB-5MS (30 m×0.25 mm i.d.; 0.25 μm; J&W Scientific, Rancho Cordova, CA, USA) was used for separation. Injections were effected automatically in the splitless mode at 260°C. The column oven temperature was maintained at 80°C for 2 min and then raised at 20°C/min to 310°C. The transferline temperature was set at 260°C. High purity helium, at a flow rate of 3 ml/min, was used as the carrier gas. The electron ionization (EI) operating parameters were as follows: source temperature, 200°C; electron energy, 70eV; ion multiplier gain, 1.2kV. Data were collected from 40-600 at a scan rate of 0.5 s/scan.

LC/MS was performed on a ZMD system equipped with an Allience 2690 pump and an electrospray ionization (ESI) interface (Waters, Milford, USA). The capillary and cone voltages were set at 2.0 kV and 25 V, respectively. The other ESI operating parameters were as follows: ion-source temperature, 100°C; desolvation temperature, 300°C; nitrogen gas, 450 l/h; and multiplier voltage, 650 V. Under these conditions, full scan data were acquired from *m/z* 100 to 600 in the centroid mode, using a cycle time of 1.0 sec and an interscan time of 0.1 sec. The quantitative measurements were accomplished in the selected ion monitoring (SIM) mode by monitoring each protonated molecules as follows: *m/z* 275 for 5-MeO-DIPT, *m/z* 233 for 5-MeO-NIPT, *m/z* 261 for 5-OH-DIPT, *m/z* 291 for 5-MeO-6-OH-DIPT, and *m/z* 175 for 5-MT, by an internal standard method. The chromatographic

separation was carried out on a semi-micro L-column ODS column (1.5 mm i.d.×150 mm; Chemicals Evaluation and Research Institute, Tokyo, Japan) with a binary mobile phase of methanol and 10 mM ammonium formate (pH 3.5), using a linear gradient (methanol 25 to 40% in 5 min). The flow rate was set at 0.1 ml/min, and the entire flow was introduced into the ESI source.

LC/MS/MS was performed on an Quattro LC (Micromass, Manchester, UK) system equipped with an Agilent 1100 pump (Agilent Technologies, Palo Alto, CA, USA) and an ESI interface under the same chromatographic conditions and operating parameters, except that the ion-source temperature was set at 280°C. Argon was used as the collision gas at collision energies of 15 eV (for conjugates of 6-OH-5-MeO-DIPT) and 20 eV (for conjugates of 5-OH-DIPT). Ions of *m/z* 341, 437, 371, and 467, corresponding to the protonated molecules of 5-OH-DIPT-sulfate, 5-OH-DIPT-glucuronide, 6-OH-5-MeO-DIPT-sulfate, and 6-OH-5-MeO-DIPT-glucuronide, respectively, were selected as precursor ions.

High-resolution MS and MS/MS were performed on an LCMS-IT-TOF (Shimadzu). The probe voltage was +4.5 kV and both the curved desolvation line temperature and the block heater temperature were 200°C. The ion accumulation time and isolation time were set at 30 msec and 20 msec, respectively. Argon was used as collision gas at 50% of introduction. The collision-induced dissociation (CID) energy and the CID time were 50% and 30 msec, respectively.

Specimen Collection.

Urine specimens were voluntarily provided from two 5-MeO-DIPT users. User A (24-year-old Japanese male) consumed a capsule containing approximately 50 mg of 5-MeO-DIPT hydrochloride through the anus (exact dosage unclear). About 30 min

later, he began intensive sweating, became hyperaggressive, and was taken to the emergency department with dementia. His urine specimens were collected at approximately 11 h and 35 h post-intake. User B (22-year-old Japanese male) drank fruits juice spiked with 5-MeO-DIPT hydrochloride (dosage unclear). He was brought to the hospital with strong dementia. The urine specimen was collected at approximately 18 h post-intake. Urine specimens were immediately frozen and stored at –20°C until analysis.

Sample Preparation.

Extraction and TMS derivatization for GC/MS

A 1 ml sample of a urine specimen was adjusted to pH 8 with 2.8% ammonium hydroxide, mixed vigorously with 500 μl of a chloroform-isopropyl alcohol mixture (3:1, v/v) and centrifuged at 1500×*g* for 10 min. The organic layer was recovered and dehydrated with anhydrous sodium sulfate. A 1-μl aliquot was injected into the GC/MS system. A 100 μl aliquot of the extract was transferred into a screw-capped glass tube and was derivatized by adding 100 μl of MSTFA and then heating at 60°C for 30 min. A 1-μl aliquot of the reaction mixture was manually injected into the GC/MS system.

Enzymatic hydrolysis

To a 300 μl urine specimen was added 30 μl of 0.1 M ascorbic acid, and the specimen was adjusted to pH 5 with 10% acetic acid. After adding 60 μl of 0.5 M acetate buffer (pH 5.0) and *H. pomatia* sulfatase/β-glucuronidase (300 and 8680 Fishman units, respectively), this was then incubated at 37°C for 2 h to hydrolyze conjugates.

Extraction for LC/MS of free-form metabolites

Urine specimens before and after hydrolysis were adjusted to pH 8 with 2.8% ammonium hydroxide and mixed vigorously with two volumes of a chloroform-isopropyl alcohol mixture (3:1, v/v). After centrifugation, the organic layers were recovered. The extraction was repeated twice, and the organic extracts were combined, and this was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 100 μl distilled water, and the I.S. solution was added. This was then filtered through a 0.2 μm membrane filter, and an aliquot of 5 μl was automatically injected into the LC/MS system.

Extraction for LC/MS and LC/MS/MS of conjugates

Urine specimens before and after hydrolysis were each mixed vigorously with two volumes of methanol. After centrifugation, the supernatant was recovered and evaporated to dryness. The residue was dissolved in 100 μl distilled water and filtered. Aliquots of 5 μl were automatically injected into the LC/MS and LC/MS/MS systems.

Validation of the LC/MS procedure.

In order to quantify 5-MeO-DIPT and its metabolites in urine, the LC/MS procedure optimized was validated. A 300-μl drug-free urine spiked with the synthesized standards at 1 μg/ml each was processed as described in the Experimental section. The residue was dissolved in 100 μl distilled water and added to 100 μl of 200 ng/ml I.S. (5-MT) solution. A 5 μl aliquot was injected into the LC/MS system in the SIM mode, where the protonated molecules of each analyte

were selected as the monitoring ions, and the peak area ratios to I.S. were calculated. The recoveries at 1 μg/ml were 93.1%, 95.7%, 92.3%, and 105% for 5-MeO-DIPT, 5-OH-DIPT, 5-MeO-NIPT, and 6-OH-5-MeO-DIPT, respectively (*n*=5). The detection limits were 0.03 μg/ml for 6-OH-5-MeO-DIPT and 0.003 μg/ml for the others. Calibration curves constructed by the I.S. method showed good linearities over the ranges from 0.1 to 10 μg/ml for 6-OH-5-MeO-DIPT and from 0.01 to 10 μg/ml for the others. The within-day relative standard deviations (evaluated at 1 μg/ml, *n*=5) ranged from 3.35 to 5.17 % for all of the analytes. These results guaranteed the reliability of the present procedure for the analysis of urine specimens from 5-MeO-DIPT users.

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Results

GC/MS and LC/MS of predicted metabolites standards

The EI mass spectra of the authentic standards, except for 5-MeO-DIPT-NO, indicated their characteristic structures; predominant ions due to the α-cleavage of amine moieties (*m/z* 72 for 5-MeO-NIPT, *m/z* 114 for 5-MeO-DIPT, 5-OH-DIPT, and 6-OH-5-MeO-DIPT) and some other ions, including a very small molecular ion (*m/z* 274 for 5-MeO-DIPT, *m/z* 232 for 5-MeO-NIPT, *m/z* 260 for 5-OH-DIPT, and *m/z* 290 for 6-OH-5-MeO-DIPT) (data not shown). 5-MeO-DIPT-NO was not detectable by GC/MS because *N*-oxide metabolites readily degrade in the injection port of the gas chromatograph.

GC/MS was also carried out after TMS derivatization because of its higher sensitivity and clearer identification of amines and phenols. The EI mass spectra of their TMS derivatives also had a molecular ion (*m/z* 346 for 5-MeO-DIPT-TMS, *m/z* 304 for 5-MeO-NIPT-TMS, *m/z* 404 for 5-OH-DIPT-*di*-TMS, and *m/z* 434 for 6-OH-5-MeO-DIPT-*di*-TMS), but their relative intensities were still very low, as in the case without derivatization (data not shown).

Unlike GC/MS spectra, the ESI mass spectra of the authentic standards taken by LC/MS (data not shown) were characterized by the predominant protonated molecules at *m/z* 275 for 5-MeO-DIPT, *m/z* 233 for 5-MeO-NIPT, *m/z* 261 for 5-OH-DIPT, *m/z* 291 for 6-OH-5-MeO-DIPT and 5-MeO-DIPT-NO. It should be noted that the ESI mass spectrum of 5-MeO-DIPT-NO had a weak dimerization ion $[2M+H]^+$, which often appears specifically for amine *N*-oxides, at *m/z* 581.

Identification of the Metabolites in Urine

The extracts of the unhydrolyzed urine specimens were first analyzed by GC/MS

with and without TMS derivatization, and the retention times and mass spectra of compounds detected were compared with those of the standards. As a result, free-form 5-MeO-DIPT, 5-MeO-NIPT, 5-OH-DIPT, and 6-OH-5-MeO-DIPT were detected in the extracts without derivatization, and TMS derivatives of these four compounds were also clearly detected in the derivatized extracts.

LC/ESI MS was next carried out under the optimized conditions, and the results were compared with those of the authentic standards. Fig. 2 shows the extracted ion chromatograms obtained from User A's urine (11 h post intake). Although 5-MeO-DIPT, 5-MeO-NIPT, 5-OH-DIPT, and 6-OH-5-MeO-DIPT were confirmed, no trace of 5-MeO-DIPT-NO was detected in this specimen.

In order to examine the stability of the predicted *N*-oxide metabolite, six control urine samples from drug-free volunteers were spiked with 5-MeO-DIPT-NO at 1 μg/ml, and the samples were stored at -20°C for one month. No noticeable decrease of 5-MeO-DIPT-NO was detected in any of the samples tested. Thus, the absence of 5-MeO-DIPT-NO was not attributed to its denaturation or decomposition.

Excretion of 5-MeO-DIPT and Its Metabolites

The concentrations of 5-MeO-DIPT and its three metabolites identified in the urine specimens were quantified by the validated LC/MS procedure, using the calibration curves constructed, and the excretion profiles were investigated. Also, the concentrations before and after enzymatic hydrolysis were compared. The results are summarized in Table 1. Although 5-MeO-DIPT, 5-MeO-NIPT, 5-OH-DIPT, and 6-OH-5-MeO-DIPT were detected in all of the samples, no trace of 5-MeO-DIPT-NO was detected in any of the specimens examined.

Because excretion of the glucuronides and sulfates were expected for

5-OH-DIPT and 6-OH-5-MeO-DIPT from their increased levels after hydrolysis, their direct detection was attempted by LC/MS/MS. Typical extracted ion chromatograms obtained before and after sulfatase/β-glucuronidase treatment, are shown in Fig. 3. Unhydrolyzed urine gave four distinctive peaks at retention times of 4.3, 3.0, 4.1, and 4.8 min on the extracted ion chromatograms at *m/z* 341, 437, 371, and 467, respectively, which correspond to the protonated molecules of 5-OH-DIPT-sulfate, 5-OH-DIPT-glucuronide, 6-OH-5-MeO-DIPT-sulfate, and 6-OH-5-MeO-DIPT-glucuronide, respectively. The sulfatase/β-glucuronidase treatment for 2 h resulted in decreases in the peak areas at 4.3 and 4.1 min, and disappearance of the peaks at 3.0 and 4.8 min, while the peak areas of 5-OH-DIPT and 6-OH-5-MeO-DIPT significantly increased. To confirm these peak assignments, these peaks were further analyzed by MS/MS. The results are summarized in Fig. 4. The results indicate that the metabolites eluting at 4.3 and 3.0 min were 5-OH-DIPT-sulfate and 5-OH-DIPT-glucuronide, as indicated by the neutral loss of 80 (the sulfate group), and that of 176 (the glucuronyl group), to produce the protonated molecule of 5-OH-DIPT (*m/z* 261) and its characteristic substructural ions (*m/z* 114 and 160). In the same manner, the metabolites eluting at 4.1 and 4.8 min were proved to be 6-OH-5-MeO-DIPT-sulfate and 6-OH-5-MeO-DIPT-glucuronide; the neutral losses of 80 and 176 resulted in the protonated molecule of 6-OH-5-MeO-DIPT (*m/z* 291) and its characteristic substructural ion (*m/z* 114).

Discussion

Identification of Metabolites by GC/MS and LC/MS

For urinary metabolites of 5-MeO-DIPT, Meatherall's team has recently reported the "tentative identification" of 5-MeO-NIPT, 5-OH-DIPT, and 5-MeO-DIPT-*N'*-oxide (Meatherall and Sharma, 2003; Wilson et al., 2005). However, their identification was based only on the GC/EI MS data without comparison with authentic standards synthesized. Especially, because *N*-oxide metabolites readily degrade as mentioned above, it is well known that *N*-oxides are normally undetectable by GC/MS. Thus, we concluded that the detection of "*N'*-oxide" (it should be named "hydroxylamine", rather than "*N'*-oxide") in their reports required reexamination by LC/MS, based on our previous studies that dealt with *N*-oxide metabolites of several other drugs (Katagi et al., 2000; Katagi et al., 2001).

Previous studies on the rat reported metabolic routes of DMT and 5-MeO-DMT, which include *N*-oxidation, *N*-demethylation, *O*-demethylation, and oxidative deamination (Sitaram et al., 1987). Based on their report, 5-MeO-NIPT, 5-OH-DIPT, and 5-MeO-DIPT-NO were predicted as the major urinary metabolites of 5-MeO-DIPT. In addition, Morano et al. (1993) proposed that etryptamine (ethyltryptamine) is metabolized mainly by 6-hydroxylation, like other indole derivatives, which suggested the possibility of hydroxylation at the 6-position of 5-MeO-DIPT into 6-OH-5-MeO-DIPT. Thus, the present authors selected 6-OH-5-MeO-DIPT, in addition to the above-mentioned three metabolites, as the expected major metabolites which retain the structural characteristics of the parent drug 5-MeO-DIPT. Based on the GC/MS and LC/MS analyses in this study, 5-MeO-DIPT, 5-MeO-NIPT, 5-OH-DIPT, and 6-OH-5-MeO-DIPT were indisputably identified in human urine from 5-MeO-DIPT users.

In this study, no trace of 5-MeO-DIPT-NO was detected in any of the urine specimens from 5-MeO-DIPT users. In the previous study by Sitaram et al. (1987) on the metabolism of 5-MeO-DMT, an analog of 5-MeO-DIPT, 5-MeO-DMT-*N*-oxide was identified as the abundant and characteristic urinary metabolite in the rat. This difference is probably attributed to steric interference with *N*-oxidation on the tertiary nitrogen atom by the two bulky isopropyl moieties, though the possibility of interspecies variations should not be excluded. Also, no noticeable decrease of 5-MeO-DIPT-NO during sample storage was detected as mentioned above. Thus, there is probably no metabolic transformation of 5-MeO-DIPT into 5-MeO-DIPT-NO in humans.

Our studies confirm that 5-MeO-NIPT and 5-OH-DIPT are metabolites found in human urine as predicted by Meatherall and Sharma (2003) and Wilson et al. (2005). However, 6-OH-5-MeO-DIPT was also identified rather than 5-MeO-DIPT-*N'*-oxide as the GC/MS product close to the 5-OH-DIPT peak. Because 5-MeO-DIPT-*N'*-oxide and 6-OH-5-MeO-DIPT have the same molecular weight, we concluded that the metabolite that Meatherall's team expected to be 5-MeO-DIPT-*N'*-oxide was probably the 6-OH-5-MeO-DIPT identified here.

Excretion of 5-MeO-DIPT and Its Metabolites

As summarized in Table 1, enzymatic hydrolysis increased 6-OH-5-MeO-DIPT concentration to almost the same level as that of 5-OH-DIPT, though no notable increase was observed for 5-OH-DIPT. These facts indicate that 5-OH-DIPT and 6-OH-5-MeO-DIPT are abundant and characteristic metabolites and that *N*-deisopropylation is a minor metabolic pathway for 5-MeO-DIPT.

Based on the quantitative results listed in Table 1, it should be noted that the

conjugation rate of 5-OH-DIPT seemed to be very low, though most of 6-OH-5-MeO-DIPT was excreted as its conjugates. For determining exact rates of sulfates and glucuronides, analysis should be done using authentic standards of conjugates, or by performing selective hydrolysis using sulfatase and β-glucuronidase inhibitors. However, in the direct analysis of the conjugates of hydroxylated metabolites of phenethylamine-type drugs by LC/ESI MS and LC/ESI MS/MS, we found that the sensitivities in detecting glucuronides were 1.2-2.3 times higher than those of sulfates in the LC/ESI MS of *p*-hydroxymethamphetamine and 4-hydroxy-3-methoxymethamphetamine, the major metabolites of methamphetamine and 3,4-methylenedioxymethamphetamine. Based on these facts, the present data in Fig. 3 suggests that 5-OH-DIPT is excreted in urine mainly in the unconjugated form, while 6-OH-5-MeO-DIPT is excreted mostly as its sulfate.

These results revealed three metabolic pathways of considerable quantitative significance for 5-MeO-DIPT in humans. As shown in Fig. 5, the first pathway leads, *via* side-chain degradation by *O*-demethylation, to 5-OH-DIPT, partly followed by conjugation to form its sulfate and glucuronide. The second pathway leads, *via* direct hydroxylation on position 6 of the aromatic ring of 5-MeO-DIPT; and/or methylation of the hydroxyl group on position 5, following hydroxylation on position 6 of the aromatic ring of 5-OH-DIPT, to produce 6-OH-5-MeO-DIPT, followed by conjugation to its sulfate and glucuronide. Though the hydroxylated 5-OH-DIPT (Fig. 5) is also expected to be methylated to form 5-hydroxy-6-methoxy-diisopropyltryptamine the positional isomer of 6-OH-5-MeO-DIPT, 6-OH-5-MeO-DIPT and 5-hydroxy-6-methoxy-diisopropyltryptamine should be distinguished by their retention times on GC/MS and LC/MS according to our previous studies: The positional

isomers of *N*-benzylpiperazine metabolites (Tsutsumi et al., 2005b) and MDMA metabolites (unpublished data) had been well separated by GC/MS and LC/MS. And the urinary hydroxy-methoxy-diisopropyltryptamine detected in this study completely agreed with 6-OH-5-MeO-DIPT. We, therefore, concluded that the hydroxylated metabolite is 6-OH-5-MeO-DIPT. The third pathway leads, *via* side-chain degradation by *N*-deisopropylation, to the corresponding secondary amine, 5-MeO-NIPT. Of these, 5-OH-DIPT and 6-OH-5-MeO-DIPT were of the major metabolites. However, further investigation of hydrolysis conditions is necessary to exactly evaluate the contributions of demethylation and hydroxylation to 5-MeO-DIPT metabolism because the hydrolysis of 5-OH-DIPT-sulfate and 6-OH-5-MeO-DIPT-sulfate could not be completed under the current conditions (Fig. 3). Also, a more quantitative experiment using radiolabeled 5-MeO-DIPT in an animal model might be very useful for more detailed survey of the metabolism of 5-MeO-DIPT. It is well known that oxidative *N*-deamination is a major metabolic pathway for 5-MeO-DMT in the rat (Sitaram et al., 1987), and thus it is expected to be an important metabolic pathway for 5-MeO-DIPT in humans. However, possible metabolites like 5-methoxyindoleacetic acid and 5-hydroxyindoleacetic acid (5-HIAA), formed by the oxidative deamination, retain few structural characteristics of the parent compound. In fact, 5-HIAA, which is commercially available, is known to be present at low μg/ml level in the urine of healthy humans. Thus, the identification of characteristic urinary metabolites of 5-MeO-DIPT reported here will be of importance in forensic and clinical urine analysis. In addition, the present study will provide useful information in the urine analysis for unknown tryptamine-type drugs that may be encountered in the future.

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Footnotes

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Legends for figures

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

Fig. 2. Extracted ion chromatograms obtained from a 5-MeO-DIPT user's urine specimen by LC/MS.

Fig. 3. Comparison of LC/MS extracted ion chromatograms obtained from a 5-MeO-DIPT user's urine specimen before (left) and after (right) enzymatic hydrolysis. The urine specimen was hydrolyzed by incubation at 37°C for 2 h with *H. pomatia* sulfatase/β-glucuronidase (300 and 8680 Fishman units, respectively).

Fig. 4. Observed fragment ions on LC/MS/MS of four conjugates in a 5-MeO-DIPT user's urine specimen. Collision energies were set at 15 eV (for conjugates of 6-OH-5-MeO-DIPT) and 20 eV (for conjugates of 5-OH-DIPT). Ions corresponding to each protonated molecule of conjugates were selected as precursor ions.

Fig. 5. Metabolic pathways of 5-MeO-DIPT in humans revealed in this study. 5-MeO-DIPT is mainly metabolized by demethylation and hydroxylation. Most of 6-OH-5-MeO-DIPT formed by hydroxylation is excreted as its conjugates.

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Table 1. Urinary levels of 5-MeO-DIPT and its metabolites in 5-MeO-DIPT users' urine specimens.

	Concentrations (µg/ml urine)			
	5-MeO-DIPT	5-MeO-NIPT	5-OH-DIPT	6-OH-5-MeO-DIPT
User A-11 h post intake				
unhydrolyzed	1.8	1.7	32	2.6
hydrolyzed	1.7	1.7	47	54
User A-35 h post intake				
unhydrolyzed	<0.01	0.029	0.71	<0.1
hydrolyzed	<0.01	0.029	0.73	0.52
User B-18 h post intake				
unhydrolyzed	0.026	0.45	2.4	n.d.
hydrolyzed	0.026	0.45	2.7	3.5

n.d.: not detected

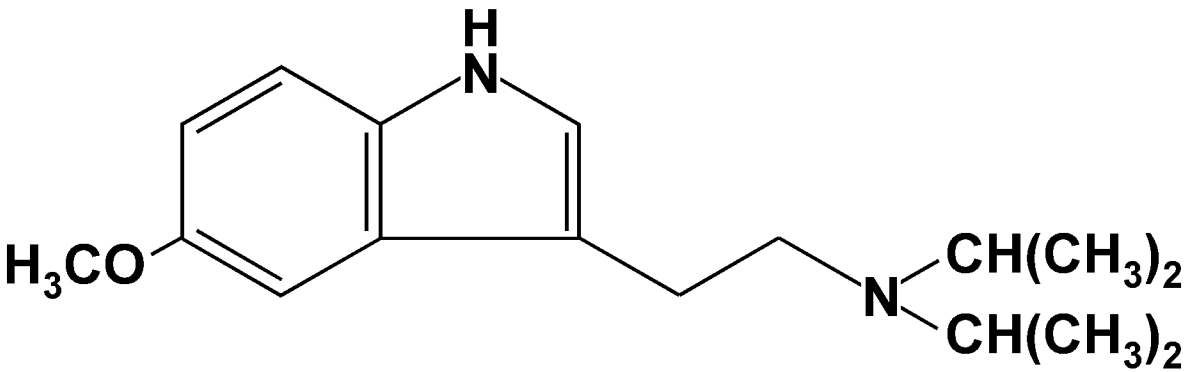


Fig. 1.

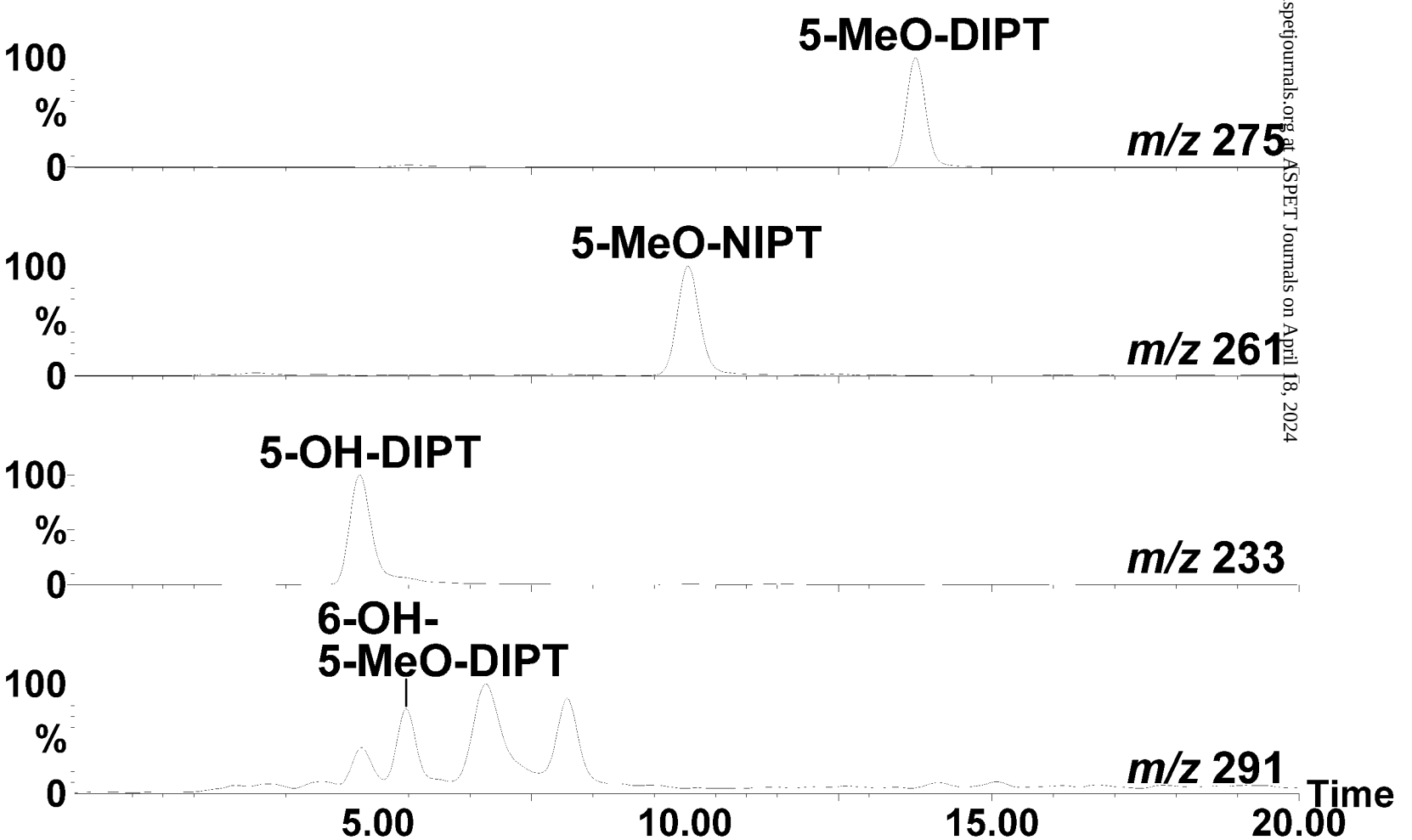


Fig. 2.

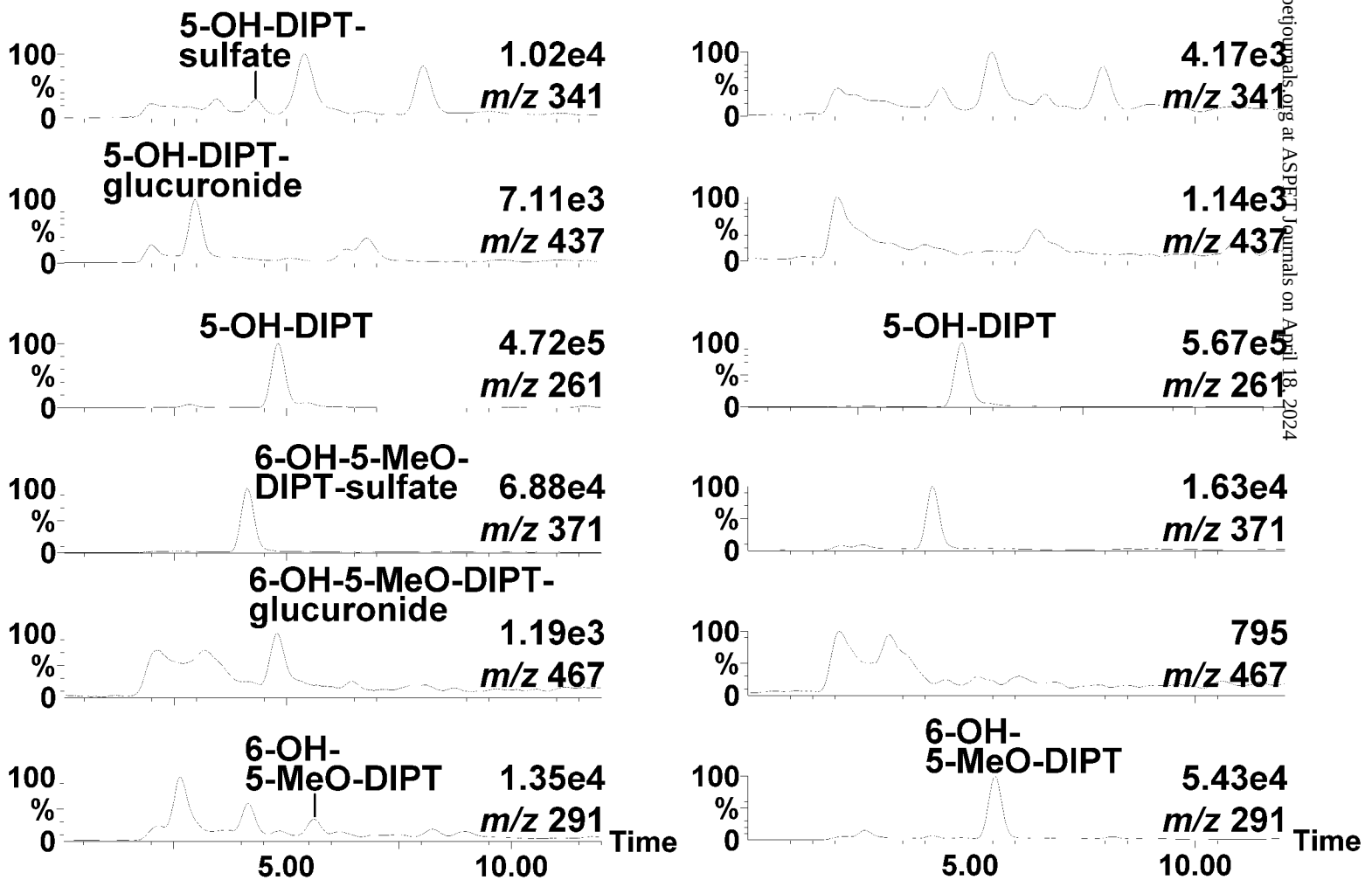
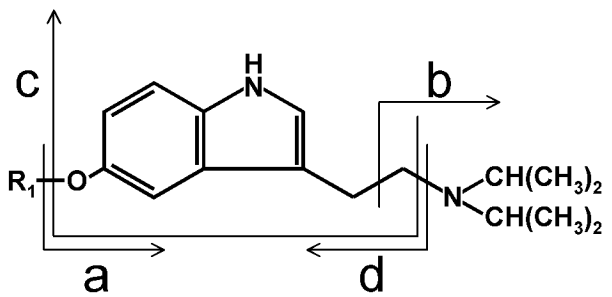
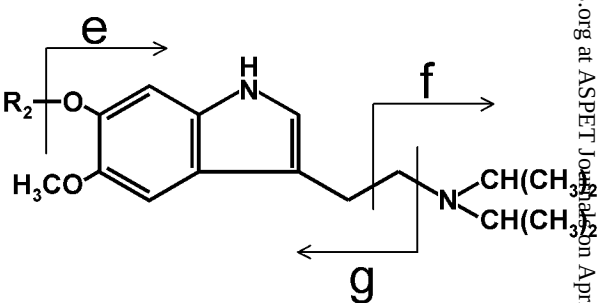


Fig. 3.





	R_1	$[M+H]^+$	a	b	c	d
5-OH-DIPT-sulfate	SO_3H	341	261	114	160	240
5-OH-DIPT-glucuronide	$C_6H_9O_6$	437	261	114	160	336

	R_2	$[M+H]^+$	e	f	g
6-OH-5-MeO-DIPT-sulfate	SO_3H	371	291	114	270
6-OH-5-MeO-DIPT-glucuronide	$C_6H_9O_6$	467	291	114	366

Fig. 4.

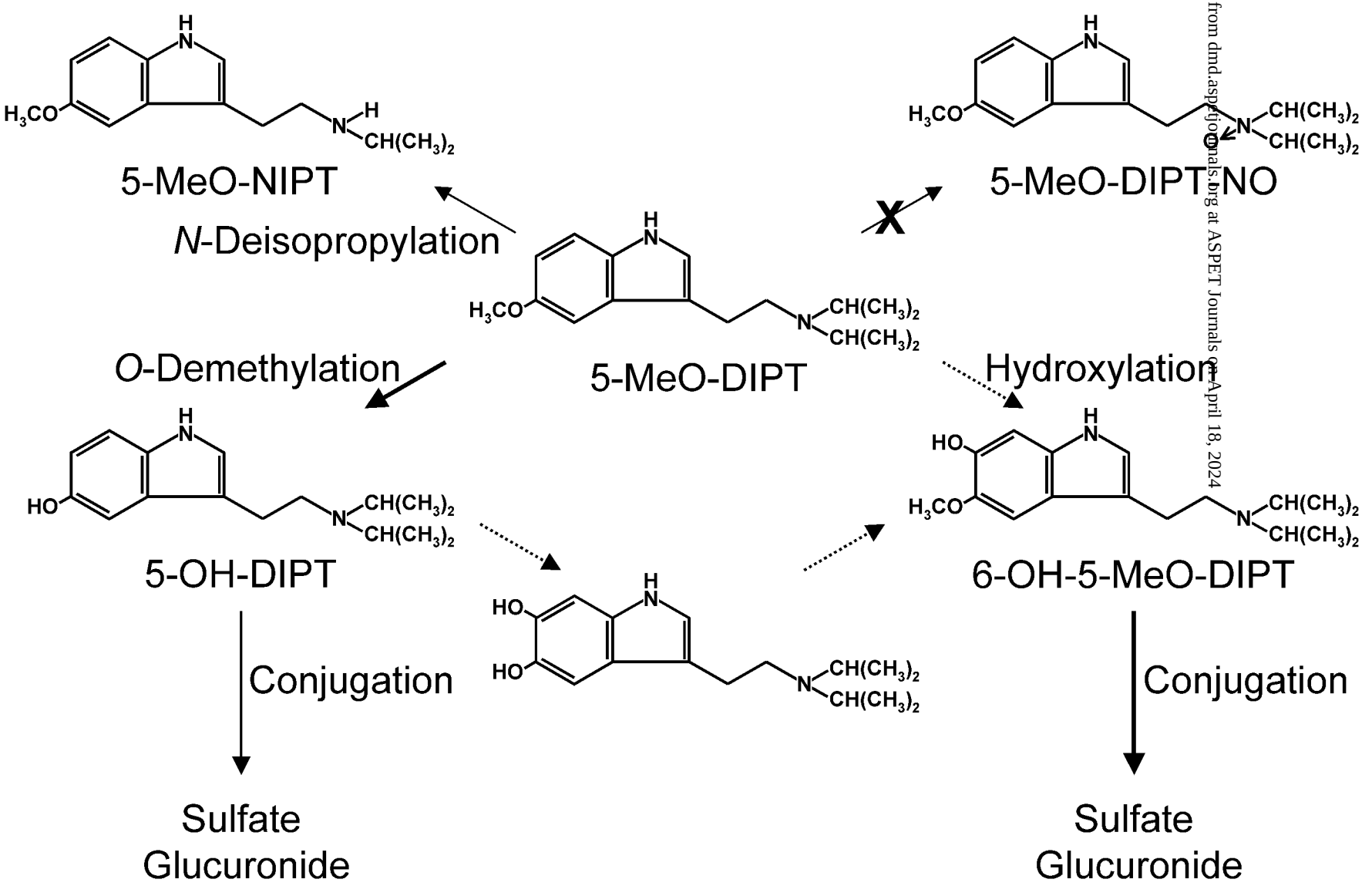


Fig. 5.