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TECHNICAL NOTE

TOXICOLOGY

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Synthesis and Identification of Urinary Metabolites of 4-Iodo-2,5-dimethoxyphenethylamine

ABSTRACT: This article describes the synthesis and identification of urinary metabolites of 4-iodo-2,5-dimethoxyphenethylamine (2C-I), a new psychoactive drug. 2C-I hydrochloride was administered orally to male Sprague-Dawley rats, and the urinary extracts were analyzed by gas chromatography/mass spectrometry (GC/MS), then five putative 2C-I metabolites were synthesized in our laboratory. In the synthetic process of the 2C-I metabolites, iodination of the aromatic ring was successfully carried out using iodine and orthoperiodic acid as the iodination reagent, and selective debenzylation of aryl benzyl ether was accomplished by the acid hydrolysis method using trifluoroacetic acid and thioanisole. The synthesized metabolites were well separated and detected by GC/MS after valeryl derivatization. The results showed that 2C-I underwent *O*-demethylation, *N*-acetylation, and deamination, followed by oxidation to the corresponding carboxylic acid in rats. The data presented in this study will be very useful for the analysis of 2C-I and its metabolites in forensic samples.

KEYWORDS: forensic science, drug of abuse, metabolite, synthesis, rat, urine, gas chromatography/mass spectrometry

4-Iodo-2,5-dimethoxyphenethylamine (2C-I, Fig. 1) is a ring-substituted phenethylamine with psychoactive properties. 2C-I is one of the "new synthetic drugs" with no therapeutic use and was first described in Shulgin's PiHKAL (1). 2C-I is typically available in powder or tablet form and, according to the user's description, the oral administration of 3–25 mg of 2C-I produces a psychedelic effect similar to 4-bromo-2,5-dimethoxyphenethylamine (2C-B), a bromine-substituted analog of 2C-I (1,2).

Today, 2C-I is a controlled drug in several countries, including Germany, the United Kingdom, Denmark, Ireland, and Greece. In Japan, 2C-I was listed as a controlled substance in 2008. In the United States of America, although 2C-I is not a scheduled drug under the Controlled Substances Act (CSA), it can be considered as an analog of 2C-B, which is in schedule I hallucinogen under the CSA, and can be restricted.

For the identification of 2C-I in biological samples, gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), and capillary electrophoresis (CE) have been used (3–6). Furthermore, Theobald et al. reported the metabolism and toxicological detection of 2C-I in rats using GC/MS and CE/MS. They identified many metabolites in rat urine and concluded that 2C-I was metabolized by *O*-demethylation, followed by *N*-acetylation or deamination, with subsequent oxidation to the corresponding acid or reduction to the corresponding alcohol, respectively (7). However, they did not confirm the identities of the metabolites using authentic standards. Therefore, the identities of the metabolites of 2C-I remain a matter for speculation.

In the present study, we synthesized the postulated metabolites of 2C-I and confirmed the presence of the metabolites in the urine of rat administered with 2C-I.

Materials and Methods

Chemicals

Authentic standards of 2C-I and its metabolites were synthesized in our laboratory. The synthetic method will be described later. β-Glucuronidase/aryl sulfatase (from *H. pomatia*; β-Glucuronidase, 5.2 units/mL; aryl sulfatase, 2.1 units/mL) was purchased from Calbiochem-Novabiochem Co. Ltd (La Jolla, CA). All other chemicals were of analytical grade.

Metabolite Synthesis

All of the synthesized standards were checked for purity and identity by thin-layer chromatography, chemical ionization mass spectrometry (CIMS), and ^{1}H-NMR. CIMS spectra were obtained using a Varian 1200L (Palo Alto, CA). Methane was used as the reagent gas. ^{1}H-NMR spectra were measured on a JEOL JNM-ECP600 (Akishima, Tokyo, Japan). Tetramethylsilane was used as an internal standard. The synthetic routes of the metabolites of 2C-I are illustrated in Fig. 2.

4-Iodo-2,5-dimethoxyphenethylamine (2C-I)

2C-I (compound #1) hydrochloride was synthesized using the method described by Shulgin et al. (1).

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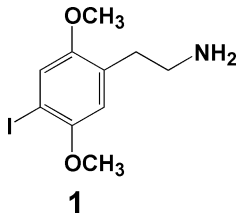

FIG. 1—*Chemical structure of 4-iodo-2,5-dimethoxyphenethylamine (2C-I).*

2-Hydroxy-4-iodo-5-methoxyphenethylamine (#6, 2-OH-2C-I)

A mixture containing 0.591 g of 2-benzyloxy-5-methoxyphenethylamine (#2, prepared by the method of Kanamori [8]) and 0.34 g of phthalic anhydride was heated at 160°C for 10 min in an oil bath. The product was purified by silica-gel column chromatography to obtain 0.598 g of *N*-(2-benzyloxy-5-methoxyphenethyl)-phthalimide (#3).

Then, 0.489 g of #3, 0.57 g of ortho-periodic acid, and 0.129 g of iodine were added to 13 mL of a mixture of acetic acid, water, and sulfuric acid (10:2:0.3, by vol.). The reaction mixture was heated at 70°C for 1 h, and, after cooling, water was added to the reaction mixture to precipitate the product. The supernatant was removed, water was added to the precipitate, the aqueous layer was made basic with sodium hydroxide solution, and extracted with chloroform. The solvent was evaporated to dryness under a vacuum, and the residue was purified by silica-gel column chromatography to give 0.553 g of *N*-(2-benzyloxy-4-iodo-5-methoxyphenethyl)-phthalimide (#4).

A solution of 0.553 g of #4 in 10 mL of 2-propanol was heated at 90°C, and 0.25 mL of hydrazine was added to the solution. After 2 h, 0.25 mL of hydrazine was added and a white solid formed. Water and chloroform were added to the reaction mixture, and the mixture was made acidic with 3 M hydrochloric acid and shaken. The aqueous layer was removed. 0.2 M hydrochloric acid was added to the remaining chloroform layer, shaken, and the aqueous layer was removed. This extraction step was repeated five times. The aqueous layers were collected, made basic using sodium hydroxide solution, and extracted with chloroform. The solvent was evaporated to dryness under vacuum to give 0.210 g of 2-benzyloxy-4-iodo-5-methoxyphenethylamine (#5) as a clear oil.

0.032 g of #5, 0.52 mL of thioanisole, and 1.6 mL of trifluoroacetic acid were stirred for 24 h. Water was added to the reaction mixture, washed with chloroform, the aqueous layer was made basic by 20% sodium carbonate solution, and extracted with chloroform. The solvent was evaporated to dryness under a vacuum to give 0.023 g of 2-hydroxy-4-iodo-5-methoxyphenethylamine (#6, 2-OH-2C-I) as a white powder. ^{1}H-NMR δ(CD_3OD): 2.75 (2H, t, J = 6.6 Hz), 2.92 (2H, t, J = 6.6 Hz), 3.76 (3H, s), 6.69 (1H, s), 7.15 (1H, s).

N-(2-hydroxy-4-iodo-5-methoxyphenethyl)-acetamide (#7, 2-OH-2C-I-NAc)

After dissolving 0.0103 g of #6 in 2.5 mL of tetrahydrofuran, 20 μL of acetic anhydride/pyridine (3:2) was added and stirred for 30 min at room temperature. After the solvent was evaporated to dryness under a vacuum, 1.5 mL of 1 M sodium hydroxide/methanol solution was added to the residue and stirred for 10 min. Water was added to the reaction mixture, the pH was made acidic with 3 M hydrochloric acid, and the sample was then extracted with dichloromethane. The solvent was evaporated to dryness under a vacuum and purified by preparative thin-layer chromatography to give 0.0083 g of *N*-(2-hydroxy-4-iodo-5-methoxyphenethyl)-acetamide (#7, 2-OH-2C-I-NAc) as a clear oil. ^{1}H-NMR δ($CDCl_3$): 2.03 (3H, s), 2.82 (2H, t, J = 7.2 Hz), 3.36 (2H, m), 3.80 (3H, s), 6.52 (1H, s), 7.33 (1H, s).

2-Methoxy-4-iodo-5-hydroxyphenethylamine (#8, 5-OH-2C-I)

Using 2-methoxy-5-benzyloxyphenethylamine (prepared using the method described by Kanamori et al. [8]) in the procedure for

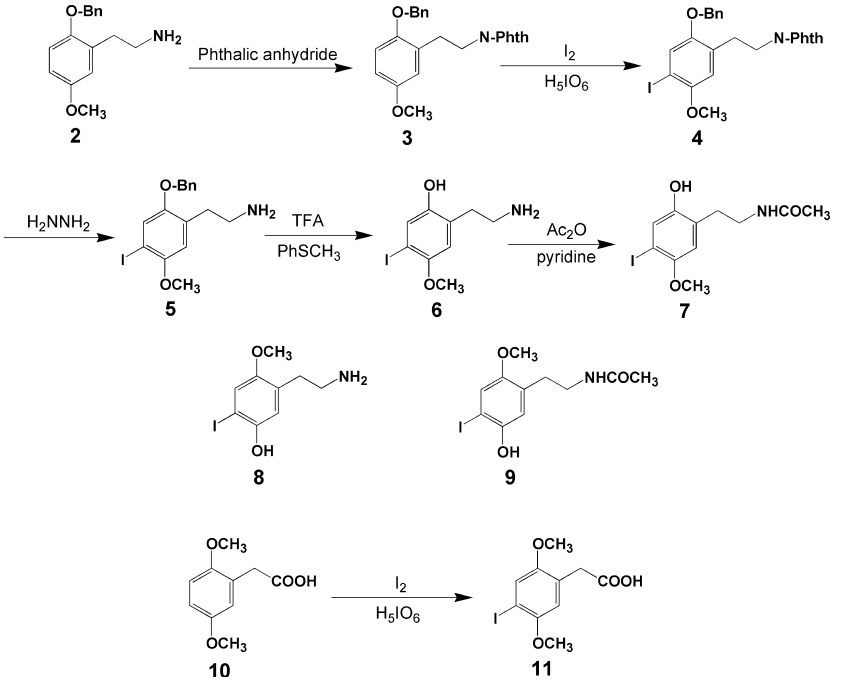

FIG. 2—*Synthetic routes for the metabolites of 2C-I.*

the synthesis of #6 gave 2-methoxy-4-iodo-5-hydroxyphenethylamine (#8, 5-OH-2C-I). ^{1}H-NMR $\delta(CD_3OD)$: 2.86 (2H, t, J = 7.5 Hz), 3.10 (2H, t, J = 7.5 Hz), 3.78 (3H, s), 6.71 (1H, s), 7.26 (1H, s).

N-(2-methoxy-4-iodo-5-hydroxyphenethyl)-acetamide (#9, 5-OH-2C-I-NAc)

Using #8 in the procedure for the synthesis of #7 gave *N*-(2-methoxy-4-iodo-5-hydroxyphenethyl)-acetamide (#9, 5-OH-2C-I-NAc). ^{1}H-NMR $\delta(CDCl_3)$: 1.94 (3H, s), 2.75 (2H, t, J = 6.9 Hz), 3.45 (2H, m), 3.78 (3H, s), 6.80 (1H, s), 7.08 (1H, s).

2,5-Dimethoxy-4-iodophenylacetic acid (#11, 2C-I-COOH)

Using 2,5-dimethoxyphenylacetic acid (#10) in the procedure for the synthesis of #4 gave 2,5-dimethoxy-4-iodophenylacetic acid (#11, 2C-I-COOH). ^{1}H-NMR $\delta(CDCl_3)$: 3.65 (2H, s), 3.80 (3H, s), 3.84 (3H, s), 6.72 (1H, s), 7.28 (1H, s).

Drug Administration and Urine Sampling

Four male Sprague-Dawley rats were orally administered with 10 mg/kg of 2C-I hydrochloride and placed in metabolic cages. Urine samples were collected over a 24-h period and stored at −30°C until used for analysis. All animal experiments were approved by the Animal Ethics Committee of the National Research Institute of Police Science.

Sample Preparation for Analysis

A few drops of acetic acid were added to a 5 mL urine sample, and the pH was adjusted to 5. Then, 100 μL of β-glucuronidase/aryl sulfatase was added to the urine, followed by incubation at 60°C for 1.5 h. After hydrolysis, the pH was adjusted to 9 by 10% sodium hydroxide solution and extracted three times with chloroform/2-propanol (3:1) (basic fraction). The remaining aqueous layer was made acidic by 3 M hydrochloric acid and extracted three times with chloroform/2-propanol (3:1) (acidic fraction). Each fraction was evaporated to dryness under a vacuum and dissolved in 1 mL of methanol.

Fifty microliters of the basic fraction was evaporated to dryness under a gentle stream of nitrogen and then dissolved in 50 μL of acetonitrile containing 5% of valeric anhydride and 5% of pyridine for gas chromatography/mass spectrometry (GC/MS) analysis. Fifty microliters of the acidic fraction was evaporated to dryness under a vacuum and then dissolved in 0.1 mL of methanol containing 10% concentrated hydrochloric acid. The resulting solution was heated at 70°C for 1 h for methylation of carboxyl group. After the reaction, the solvent was evaporated to dryness under gentle stream of nitrogen and dissolved in 50 μL of methanol for GC/MS analysis.

GC/MS

GC/MS analysis was performed using a QP-2010 plus gas chromatograph–mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a J&W DB-5ms capillary column (30 m × 0.32 mm i.d., 0.25-μm film thickness). The oven temperature was held at 100°C for 1 min after injection and then increased to 280°C at a rate of 15°C/min. The injection port and interface temperature were 250°C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The MS operated in the electron ionization mode with an ionization energy of 70 eV. The samples were injected at splitless mode (purge on time; 1.0 min).

Results and Discussion

Synthesis of the Metabolites

The *O*-demethylated metabolites of 2C-I were synthesized by the following reactions: protection of amino group by phthaloylation, iodination of the aromatic ring, dephthaloylation, and *O*-debenzylation. Iodination of the aromatic ring was successfully carried out using iodine and orthoperiodic acid as the iodination reagent (9). The use of iodine monochloride as the iodination reagent (1) resulted in the formation of a chlorinated by-product. Debenzylation of aryl benzyl ether is generally performed by catalytic reduction using palladium carbon as a catalyst; however, deiodination occurred at the same time using this method. Therefore, we tried the acid hydrolysis method using trifluoroacetic acid and thioanisole (10), and accomplished selective debenzylation of aryl benzyl ether.

GC/MS Analysis of the Authentic Standards of 2C-I and Its Metabolites

For simultaneous analysis of 2C-I and its metabolites, except for 2C-I-COOH, by GC/MS, various derivatization methods were tested. Perfluoroacylation and silylation were not suitable for the derivatization of 2C-I metabolites, because these derivatization methods resulted in the disappearance of peaks for some metabolites or the formation of by-products. On the other hand, acylation of these compounds resulted in the formation of one acylated peak for each compound. Figure 3 shows the total ion current chromatograms (TIC) obtained from GC/MS analysis of 2C-I and its four metabolites after acylation using various acylation reagents. Valeryl derivatization gave the best separation of five peaks (Fig. 3*D*); therefore, this derivatization method was adopted in this study. The TIC, mass chromatograms, and mass spectra of the valeryl derivatives of 2C-I and its metabolites are shown in Fig. 4.

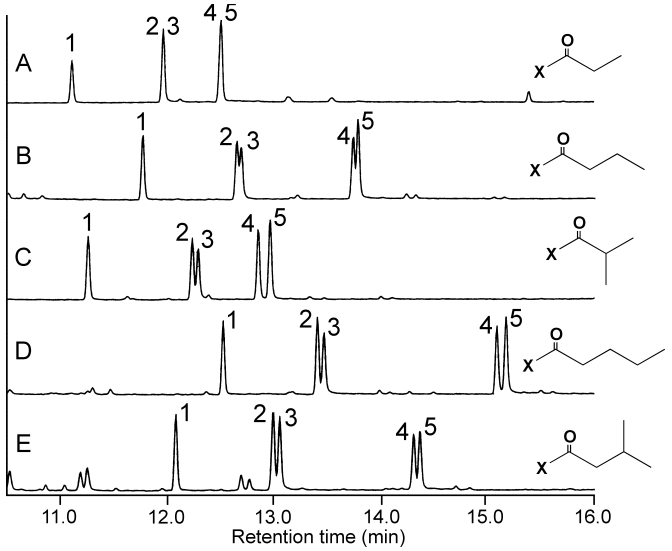

FIG. 3—*Total ion current chromatograms obtained from the acyl derivatives of 2C-I and its metabolites. (A), propionyl derivative; (B), butyryl derivative; (C), isobutyryl derivative; (D), valeryl derivative; (E), isovaleryl derivative. 1, 2C-I; 2, 2-OH-2C-I-NAc; 3, 5-OH-2C-I-NAc; 4, 2-OH-2C-I; 5, 5-OH-2C-I.*

2C-I-COOH could be analyzed by GC/MS with high detection sensitivity after methylation. Silylation was not suitable for the detection of 2C-I-COOH in rat urine because of the formation of numerous interfering peaks. The TIC, mass chromatogram, and mass spectrum for the methyl derivative of 2C-I-COOH are shown in Fig. 5.

Identification of the Urinary Metabolites

The TIC and mass chromatograms obtained from the valeryl derivatives of the basic fraction are shown in Fig. 6*A*. On the mass chromatogram of *m/z* 290, a tiny peak was detected at 12.5 min, which was considered to be the valeryl derivative of unmetabolized 2C-I. However, we could not confirm this peak because the peak was too small to obtain its mass spectrum. On the mass chromatogram of *m/z* 276, two large peaks and two small peaks were detected at 13.4–13.5 min and 15.0–15.2 min, respectively. The retention times and mass spectra of the former peaks are identical to those of the valeryl derivatives of 2-OH-2C-I-NAC and 5-OH-2C-I-NAc, and the retention times and mass spectra of latter peaks are identical to those of *di*-valeryl derivatives of 2-OH-2C-I and 5-OH-2C-I (Figs 4 and 6).

The TIC and mass chromatogram obtained from the methyl derivative of the acidic fraction are shown in Fig. 7. On the mass chromatogram of *m/z* 336, a peak was detected at 7.5 min, and the retention time and mass spectrum of this peak are identical to those of the methyl derivative of 2C-I-COOH (Figs 5 and 7).

The TICs and mass chromatograms obtained from the extract of the unhydrolyzed urine were almost identical to those of the hydrolyzed urine (data not shown), indicating that the metabolites of 2C-I hardly underwent glucuronidation or sulfoconjugation.

The proposed metabolic pathway for 2C-I in rats is shown in Fig. 8. The metabolic pathways of 2C-I include *O*-demethylation, *N*-acetylation, and deamination, followed by oxidation to the corresponding carboxylic acid.

We previously reported that 2C-B also underwent *O*-demethylation, *N*-acetylation, and deamination followed by oxidation in rats

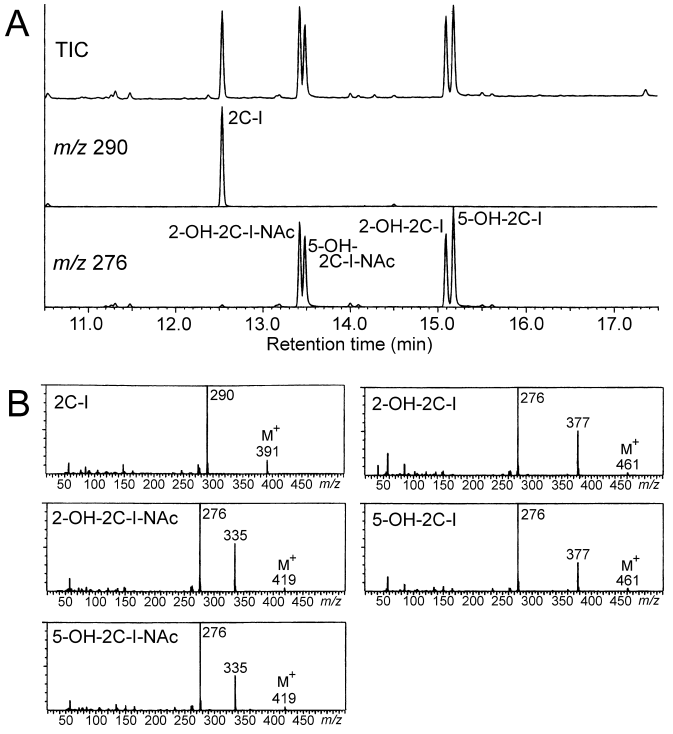

FIG. 4—*Total ion current chromatogram, mass chromatograms (A), and mass spectra (B) obtained from the valeryl derivatives of the authentic standards of 2C-I and its metabolites.*

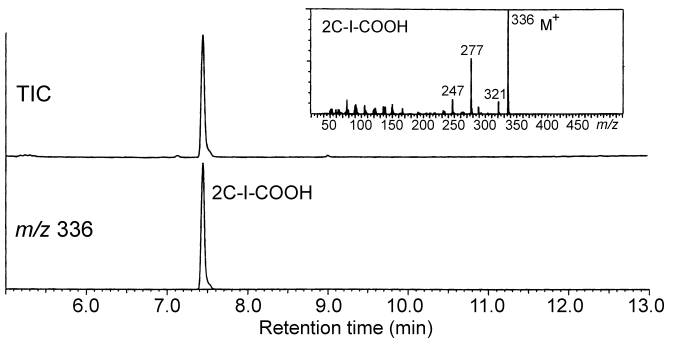

FIG. 5—*Total ion current chromatogram, mass chromatogram, and mass spectrum obtained from the methyl derivative of the authentic standard of 2C-I-COOH.*

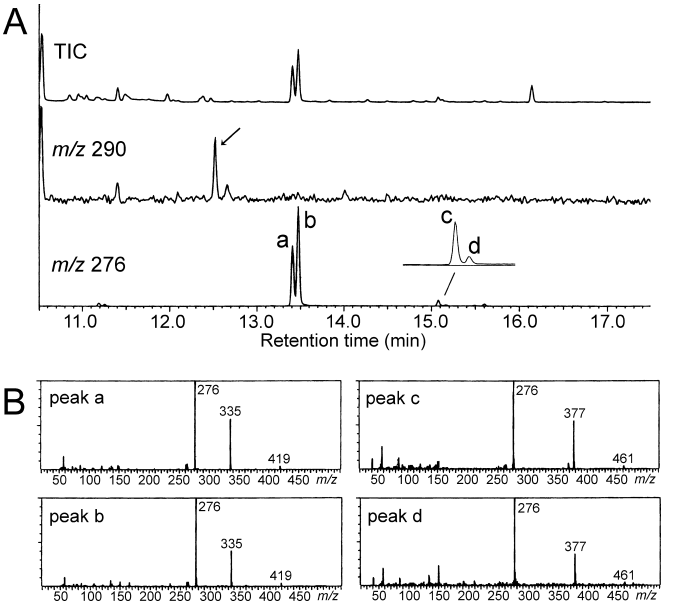

FIG. 6—*Total ion current chromatogram and mass chromatograms obtained from the valeryl derivative of the rat urine extract (basic fraction) (A) and the mass spectra for peaks a–d (B).*

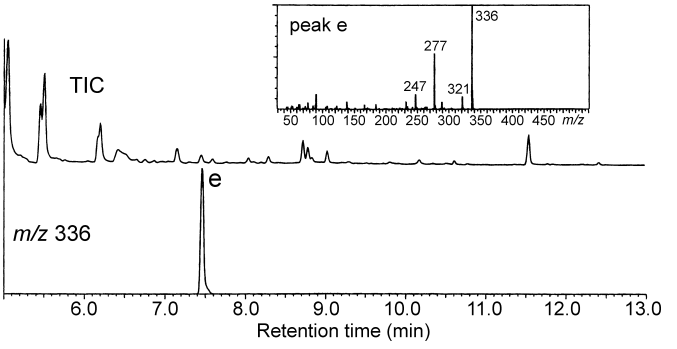

FIG. 7—*Total ion current chromatogram and mass chromatogram obtained from the methyl derivative of the rat urine extract (acidic fraction), and the mass spectrum for peak e.*

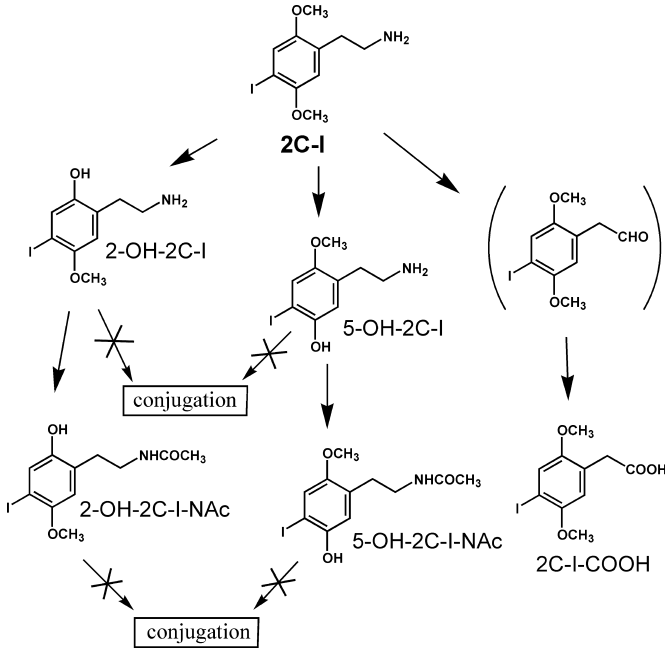

FIG. 8—*Proposed metabolic pathway for 2C-I in rats.*

(8,11). While precise quantitative analysis of the 2C-I metabolite was not performed in the present study, considering the amount of each metabolite, the peak areas of 5-OH-2C-I-NAc and 2-OH-2C-I were larger than those of 2-OH-2C-I-NAc and 5-OH-2C-I, respectively. This is in good accordance with findings for 2C-B. An alcohol compound formed by the deamination of 2C-B followed by reduction was also detected as a metabolite of 2C-B; however, we could not find a corresponding metabolite for 2C-I in this study.

The peak areas of *O*-demethyl 2C-Is and *O*-demethyl-*N*-acetyl 2C-Is were constant, regardless of the hydrolysis step, indicating that these metabolites hardly underwent conjugation. In contrast, most of the corresponding metabolites of 2C-B undergo conjugation in rats (11). It is not clear why 2C-I and 2C-B show differing susceptibility to undergo conjugation, although the difference in the atomic radius of iodine and bromine may offer one explanation.

Theobald et al. (7) reported the metabolism and toxicological detection of 2C-I in rat urine using GC/MS and CE/MS. They also detected the metabolites identified in the present study, as well as *O*-demethyl-2C-I-COOH, an alcohol compound, and β-hydroxy and β-oxo forms of the alcohol compound. However, they did not confirm the metabolites using authentic standards, and the identities of some metabolites remain a matter for speculation.

In summary, we synthesized and confirmed the metabolites of 2C-I in biological samples for the first time in this study. The data presented here will be very useful for the analysis of 2C-I and its metabolites in forensic samples.

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