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Identification of 2,5-dimethoxy-4-ethylthiophenethylamine and its metabolites in the urine of rats by gas chromatography–mass spectrometry

Li-Chan Lin [a], Ju-Tsung Liu [b], Shiu-Huey Chou [c], Cheng-Huang Lin [a,*]

[a] *Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei 116, Taiwan*
[b] *Forensic Science Center, Command of the Army Force of Military Police, Department of Defense, Taipei, Taiwan*
[c] *Department of Life Science, Fu-Jen University, 510 Chung Cheng Road, Hsinchuang, Taipei Hsien 24205, Taiwan*

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Abstract

A simple and specific method based on gas chromatography–selected ion monitoring-mass spectrometry (GC–SIM-MS) for the analysis of in vivo metabolism of 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) in rats is described. Three male rats were administered 20 mg/kg of 2C-T-2 by intra-peritoneal injection, and 24 h urine fractions were collected before and after the administration for analysis. After acidic hydrolysis of the urine samples, the metabolites were liquid–liquid extraction and analyzed by a quadruple mass spectrometer in the selected ion monitoring mode. The findings show that four metabolites of 2-(4-ethylthio-2,5-dimethoxyphenyl)-ethanol (M_w: 242), 4-ethylthio-2,5-dimethoxyphenyl acetic acid (M_w: 256), 1-acetoamino-2-(2-hydroxy-4-ethylthio-5-methoxyphenyl)-ethane (M_w: 269) and 1-acetoamino-2-(2-methoxy-4-ethylthio-5-hydroxyphenyl)-ethane (M_w: 269) are present and the metabolic pathway for 2C-T-2 in the rat is proposed.

Keywords: 2,5-Dimethoxy-4-ethylthiophenethylamine

1. Introduction

β-Phenethylamine is a natural biochemical found in both plants and animals. The increased availability of β-phenethylamine derivatives in the illicit market have became a serious social problem in many countries. Thus far, Shulgin and Shulgin [1] systematically summarized synthesis methods, dosage conditions, qualitatively described 179 phenethylamines, including 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) [2,3], 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7), etc. These compounds are often referred to as "designer drugs" because all are considered potent stimulants of the central nervous system, but contain slightly different functional groups in their chemical structure. 2C-T-2 is a ring-substituted phenethylamine, possesses psychoactive properties [1] and similar to 2C-B [4–7], but little information is available concerning its detection and metabolic fate.

In this study, we report on a simple and specific method for the detection of 2C-T-2 and its major metabolites in urine samples, after administration to rats, using gas chromatography–selected ion monitoring-mass spectrometry (GC–SIM-MS). The extraction procedures used for the urine samples, specific fragmentations for the SIM mode and several detection parameters were optimized and these data are reported herein.

2. Experimental

2.1. Reagents

Ethyl acetate, methanol, diethylether, 1,4-dimethoxybenzene, chlorosulfonic acid, zinc, sulfuric acid, ethyl bromide, potassium hydroxide, *N*-methylformanilide, nitromethane, ammonium acetate anhydrous, were all purchased from Acros (NJ, USA). Hydrochloride was acquired from Tedia company Inc. (OH, USA). Anhydrous sodium sulfate and sodium hydroxide were purchased from Yakuri Pure Chem-

* Corresponding author. Tel.: +886-2-8931-6955; fax: +886-2-2932-4249.
E-mail address: chenglin@cc.ntnu.edu.tw (C.-H. Lin).

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icals Co. Ltd. (Osaka, Japan) and J.T. Baker (NJ, USA), respectively.

2.2. Apparatus

Nuclear magnetic resonance (NMR; Bruker, Avance 500 MHz) and infrared (IR; Perkin-Elmer, FT-IR Paragon 500) spectrometry were used for the identification of products produced during different synthesis processes and for the final product. A gas chromatograph (GC 6890 Hewlett-Packard, Avondale, PA, USA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass selective detector) was used in the detection and metabolism study. A capillary column (30 m × 0.25 μm i.d.) with an HP-5MS (cross-linked 5% PH ME siloxane) bonded stationary phase film 0.25 μm thickness (Agilent Technologies, USA) was used. The inlet temperature was maintained at 250 °C. The column oven was held at 80 °C for 1 min, then programmed from 80 to 280 °C at 20 °C/min and, finally, held for 5 min. Helium at a constant flow-rate of 1 ml/min was used as the carrier gas. Data were collected using the Hewlett-Packard Chem-Station software. The mass spectrometry conditions were as follows: SIM mode; ionization energy, 70 eV; ion source temperature, 230 °C. The mass selective

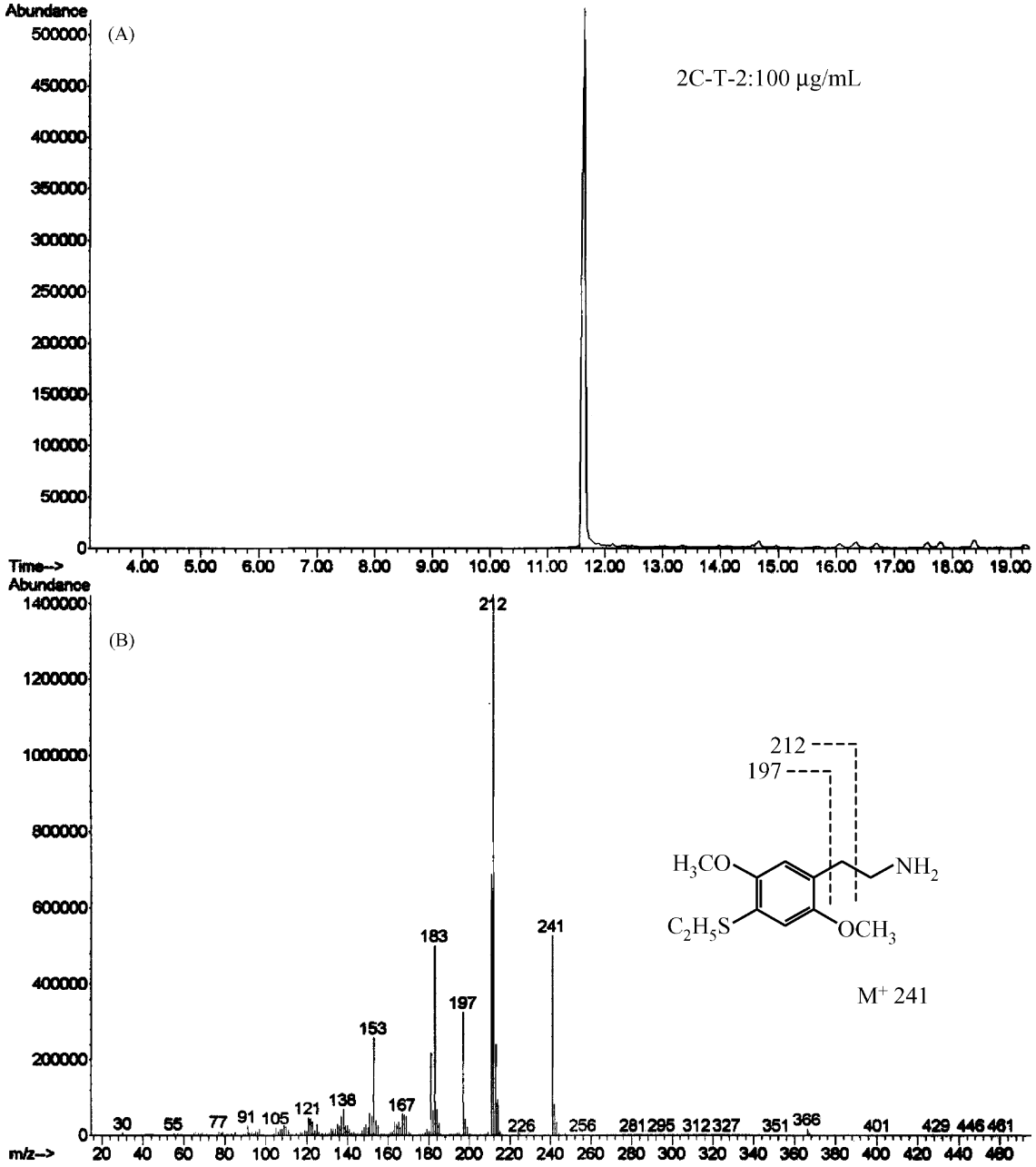

Fig. 1. (A) TIC chromatogram of 2C-T-2 (100 μg/ml in EtOH) as analyzed by GC–MS in the electron impact mode. (B) The detected peak, with a retention time of 11.72 min, permitted the specific characterization of 2C-T-2 (*m/z* of 153, 183, 197, 212 and 241). Inset: the chemical structure of 2C-T-2.

detector was operated in the SIM mode at a scan rate of 1.25 scans/s.

2.3. Drug administration and urine sampling

Three male Wistar rats were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Blank urine samples were collected at 24 h before administration. An intra-peritoneal injection (20 mg/Kg 2C-T-2 for each rat) was then performed and, following this, the rats were placed in metabolic cages for another 24 h. All urine samples were collected over a 24 h period and stored at −20 °C until used.

2.4. Extraction

Urine (1 ml) was hydrolyzed by adding 0.1 ml of 12 M hydrochloric acid at 90 °C for 30 min. When the hydrolyzed urine was cooled to room temperature, half of the hydrolyzed urine sample was directly extracted with 3 ml diethylether. This step is helpful for detecting metabolites of 2C-T-2 in the urine samples. The pH of the other half, adjusted to pH 9.5 with 2 M sodium hydroxide, and also extracted with 3 ml of diethylether. This treatment is useful for detecting 2C-T-2. The two organic layer extracts were dried over anhydrous sodium sulfate and evaporated to dryness under vacuum, respectively. Finally, the residues were dissolved in 100 μl of ethylacetate/methanol (90:10 (v/v)) for the following GC–MS experiments.

3. Results and discussion

3.1. Synthesis and GC–SIM analysis for 2C-T-2

The synthetic procedures for 2C-T-2 have been already described in the literature [1]. Followed these processes, the final product was identified by NMR, IR and then by GC–MS. Fig. 1(A) shows the total ion current (TIC) chromatogram of the final product (2C-T-2, 100 μg/ml) as analyzed by GC–MS in the electron impact (EI) mode. The detected peak, with a retention time of 11.72 min permitted the specific characterization of 2C-T-2; the presence of specific fragments, such as *m*/*z* of 153, 183, 197, 212 and 241, as shown in Fig. 1(B), permitted this characterization. The inset shows the chemical structure of 2C-T-2 and the major possible fragments (*m*/*z* of 197, 212 and 241). Furthermore, this product was of sufficient purity to be used as a standard for the further experiments. In order to understand and characterize these peaks, we used commercially available computer software (Mass Frontier 1.0), obtained from HighChem Ltd. (http://www.highchem.com, Slovakia), to perform some calculations and predictions. All of the possible fragmentation mass spectra were automatically predicted and suggested by this program when the chemical structure of 2C-T-2 was entered (in the EI mode). Based on the computer calculation, numerous situations were found to be possible. A portion of the predicted fragmentation pattern of 2C-T-2 at *m*/*z* of 153, 183 is shown in Fig. 2. As a result, we selected five prominent

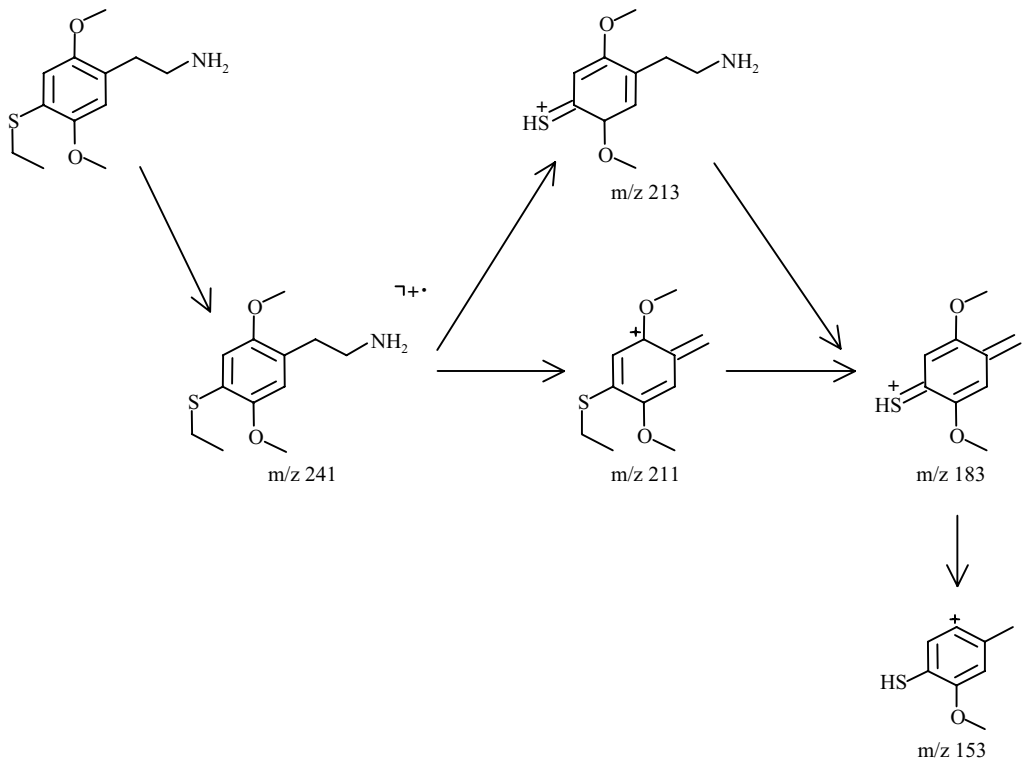

Fig. 2. Fragmentation pattern (*m*/*z* of 153, 183) of 2C-T-2 obtained by Mass Frontier 1.0.

fragmentation peaks (*m*/*z* of 153, 183, 197, 212 and 241) as markers.

3.2. Analysis of rat urine samples

Fig. 3(A) shows the TIC chromatogram for the one of the urine samples, to which 2 M sodium hydroxide was added for pH adjustment (pH = 1), and extraction by diethylether, as described above. A minor peak was detected with the same retention time (11.72 min) as the standard. Due to the combination of the mass spectrum of this peak and its retention time and these specific characterizations (*m*/*z* of 153, 183, 197, 212 and 241), we assigned this peak as the 2C-T-2 parent drug in urine and its concentration was calculated to be ~0.1 μg/ml. The inset shows the chromatographic separation of this 2C-T-2 parent drug,

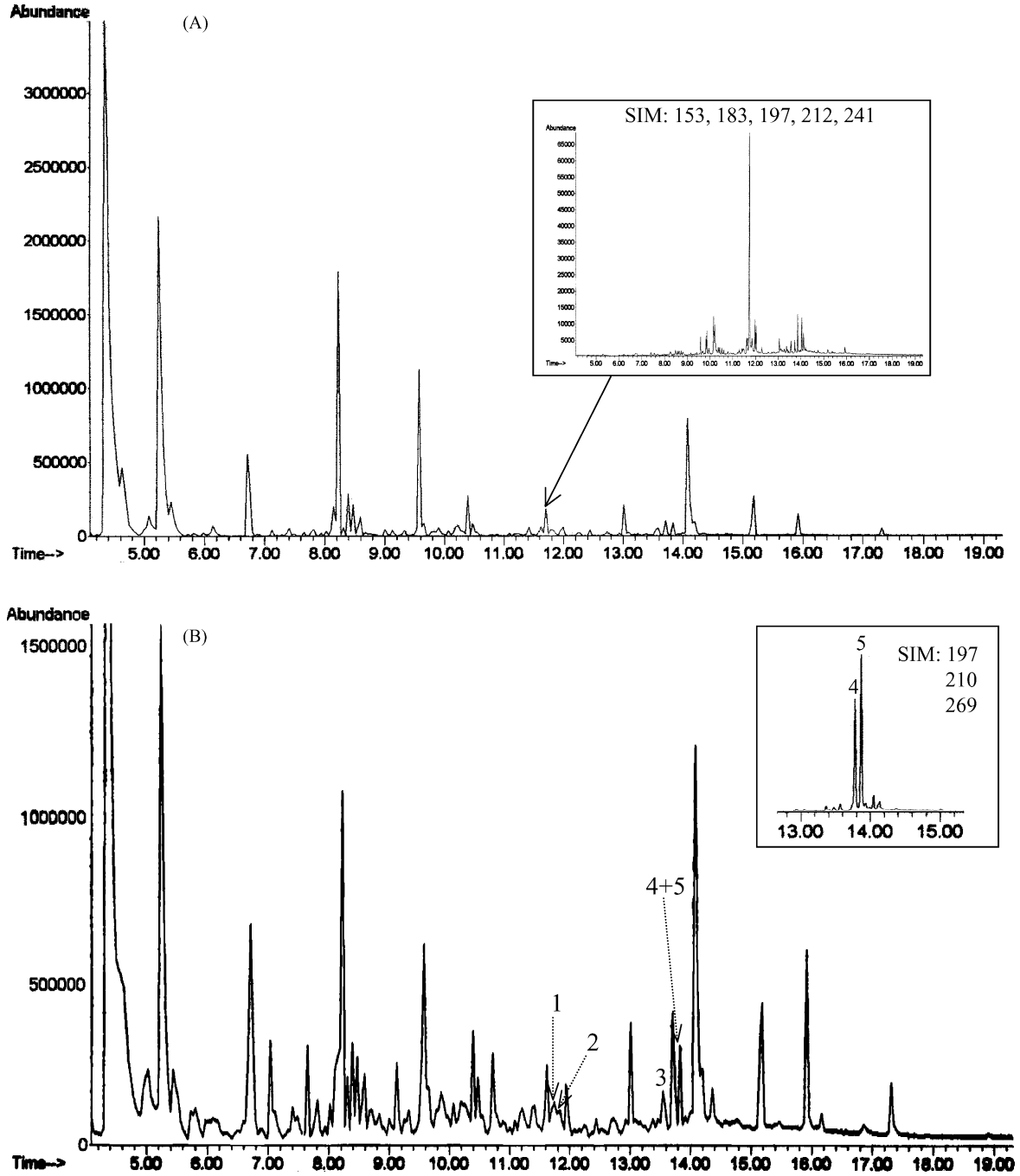

Fig. 3. (A) TIC chromatogram of a rat urine extract (adjusted to pH 9.5 with 2 M sodium hydroxide) in the electron impact mode. Inset: the SIM (*m*/*z* of 153, 183, 197, 212 and 241) chromatogram of rat urine extract. (B) TIC chromatogram of the rat urine extract (without pH adjustment). Peaks 1–5: candidates of the unchanged 2C-T-2 and its metabolites. Inset: SIM (*m*/*z* of 197, 210 and 269) chromatogram of the rat urine extract which provided two individual peak (M_w: 269) for the isomer of 1-acetoamino-2-(2-hydroxy-4-ethylsulfanyl-5-methoxyphenyl)-ethane and 1-acetoamino-2-(2-methoxy-4-ethylsulfanyl-5-hydroxyphenyl)-ethane.

which appeared as a single peak when the SIM profile was selected. Using the same procedures, similar results were obtained for the other compounds as well. However, this peak was not detected in blank urine samples. This also provides support that 2C-T-2 was not formed after deconjugation by a hydrolysis process because all urine specimens were hydrolyzed with concentrated hydrochloride acid. This information is useful for detecting 2C-T-2, which is considered to be an abuse drug, in complicated samples such as urine or blood samples from humans.

Fig. 3(B) shows the TIC chromatogram for the same rat urine sample, but without any 2 M sodium hydroxide treatment. After searching the mass spectral database and comparing these data, we selected five peaks (peaks 1–5; with retention times of 11.72, 11.83, 13.54, 13.70 and 13.82 min) for the following discussion. Peak 1 has the same retention time as the standard, but its mass spectrum is different from 2C-T-2. Thus, this peak was not assigned as the 2C-T-2 parent drug. This is also the reason why the urine sample was separated into two parts for the extractions. Peaks 4 and 5 have the same molecular weight

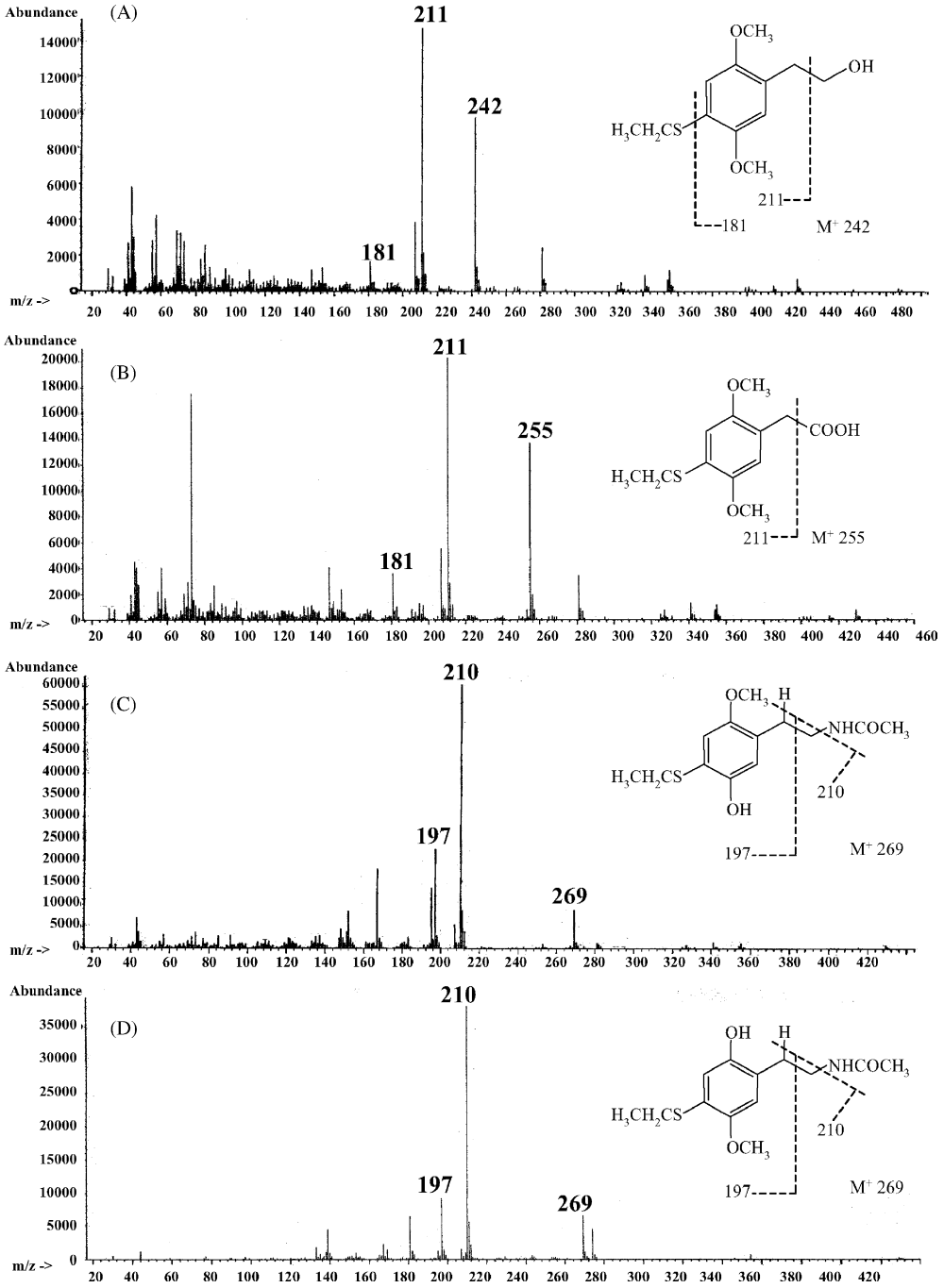

Fig. 4. (A–D) Mass spectra and their possible fragments of the peaks (2–5) appeared in Fig. 3(B), respectively.

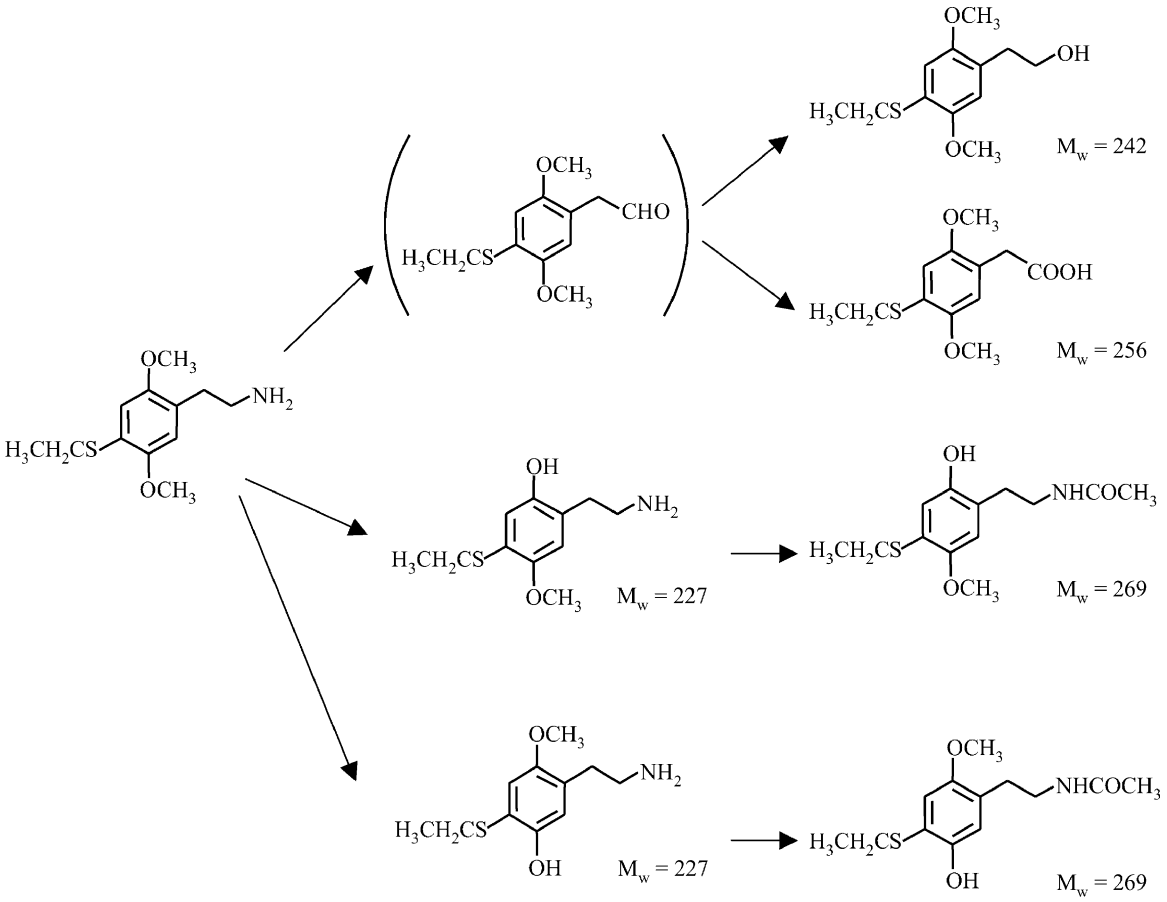

Fig. 5. Proposed metabolic pathway for 2C-T-2 in the rat.

(M_w: 269) and are incompletely separated on the TIC chromatogram. However, when the SIM mode was selected at *m/z* of 197, 210 and 269, two individual peaks appeared, as shown in the inset. Because of the unavailability of standards for spiking, distinguishing them further is not possible and peaks 4 and 5 may be switched. The mass spectra of peaks 2–5 are shown in Fig. 4(A–D). Based on these fragment patterns and calculations and predictions by the computer program (Mass Frontier 1.0), we assigned these peaks (2–5) as 2-(4-ethylthio-2,5-dimethoxyphenyl)-ethanol (M_w: 242), 4-ethylthio-2,5-dimethoxyphenyl acetic acid (M_w: 256), 1-acetoamino-2-(2-hydroxy-4-ethylthio-5-methoxyphenyl)-ethane (M_w: 269) and 1-acetoamino-2-(2-methoxy-4-ethylthio-5-hydroxyphenyl)-ethane (M_w: 269), respectively, as the metabolites of 2C-T-2 in rats. None of these peaks (1–5) were present in the blank urine samples. This supports the view that these compounds can be considered to be metabolites of 2C-T-2 and would be useful for the analysis of urine samples from drug abusers.

3.3. Proposed metabolic pathway for 2C-T-2 in the rats

Fig. 5 shows the proposed metabolic pathway for 2C-T-2 in the rat. This pathway is similar to 2C-B [6] and these findings also propose the existence of two major metabolic pathways for 2C-T-2 in rats. In the first pathway, 2C-T-2 forms an aldehyde metabolite by deamination. The intermediate (4-ethylthio-2,5-dimethoxyphenyl acetaldehyde) was not found in the TIC chromatogram because it may subsequently be reduced or oxidized to the corresponding alcohol and carboxylic acid metabolites, as shown in Fig. 5 (M_w: 242 and 256, respectively). In the second pathway, 2C-T-2 forms 2-(2-amino-ethyl)-5-ethylthio-4-methoxy phenol (M_w: 227) and 5-(2-amino-ethyl)-2-ethylthio-4-methoxy phenol (M_w: 227), and the amino groups are then acetylated to form the final metabolites, 1-acetoamino-2-(2-hydroxy-4-ethylthio-5-methoxyphenyl)-ethane (M_w: 269) and 1-acetoamino-2-(2-methoxy-4-ethylsulfanyl-5-hydroxyphenyl)-ethane (M_w: 269). However, these two compounds (M_w: 227) were not identified in this study. A quantitative evaluation of these metabolites and its relevance to medicinal chemistry are currently in progress.

4. Conclusions

A simple and specific method based on gas chromatography–selected ion monitoring-mass spectrometry was developed for the detection of unchanged 2C-T-2 and for the analysis of its in vivo metabolism in rats for the first time.

The 2C-T-2 parent drug in extracts of rat urine appeared as a major peak by chromatographic separation when five prominent fragmentation peaks (*m*/*z* of 153, 183, 197, 212 and 241) were selected as markers. Four major metabolites of 2C-T-2 were identified by this method. Collectively, this information is useful for the identification and quantitation of 2C-T-2 in humans in the future.

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