A Smoking Case in which Dimethylamphetamine as a Pyrolysis Product of Methamphetamine (MA) was Detected in the Urine of MA Abuser

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(Received February 24, 2004; Accepted April 14, 2004)

There had been a smoking case in which dimethylamphetamine (DMA) and its metabolite DMA-N-oxide (DMAO) in addition to methamphetamine (MA) and amphetamine (AM), were detected in the abuser's urine. The analytical results of the confiscated leftover drug enabled us to consider that DMA detected in the abuser's urine would be the pyrolysis product formed by smoking MA. In this study, we carried out a smoking experiment based on this smoking case, and then gave the case full consideration. We heated MA hydrochloride in the range of 250°C to 350°C, in which demethylation and methylation reactions occurred mainly, with a smoke collection apparatus and a gas lighter, and trapped the generating vapor with an adsorption cartridge. AM and DMA were produced *via* the demethylation and methylation reactions of a methylamino group of MA. Allylbenzene, benzaldehyde, *cis*-β-methylstyrene, benzyl chloride and *trans*-β-methylstyrene were also formed as pyrolysis products. The sum of the formation ratio of DMA to the starting MA in the cartridge eluate and that in the residual materials inside the smoke collection apparatus was 4.98%. The adsorption of some pyrolyzates containing DMA on the cartridge means that these pyrolyzates can be taken into an abuser's body. The ratios of DMA to AM in the smoking experiments and the smoking case were 52.3% and 50.3%, respectively, and they were nearly equal. There will be no inconsistencies in considering that AM and DMA have been formed as pyrolysis products of MA in the smoking case, and DMA and its metabolite DMAO have been detected in the urine of this smoking abuser.

Key words —— methamphetamine, dimethylamphetamine, amphetamine, dimethylamphetamine-N-oxide, pyrolysis product, smoking

INTRODUCTION

Methamphetamine (MA) is usually self-administered through intravenous injection; however, it can be orally smoked or ingested. In most smoking cases, abusers place the drug on a piece of aluminum foil and inhale the vapors released by heating it with a lighter — usually through a straw or a similar device. In some smoking cases, abusers make use of a glass tube, light bulb, or glass pipe instead of aluminum foil. Cook *et al*. found that MA plasma levels by smoking remained high for a considerable period of time before declining with a half-life of about 12 hr.[1] They suggest that this long plateau effect and the half-life of MA are considerably dangerous to smoking abusers in repeated smoking of MA since markedly higher plasma concentrations could be expected to occur if the dose is repeated even at fairly long intervals. Matsumoto *et al*. determined the differences in life backgrounds and clinical features between MA smokers and injectors in Japan.[2] They conclude that smoking MA does not appear to be a safer route than injection as regards losing control of MA use and inducing psychosis.

In a personal communication, K. H. Davis found that 91% of MA hydrochloride (MA-HCl) was recovered unchanged after volatilization at 300°C in a tube furnace, 81% at 400°C, 62% at 600°C and 38% at 800°C.[3] Sekine and Nakahara examined the pyrolysis products of MA-HCl by gas chromatography-mass spectrometry (GC-MS) system connected with a Curie point pyrolyzer, and found several pyrolysis products containing dimethylamphetamine (DMA) to be formed at 358°C.[4] In the previous report,[5] we clarified several pyrolysis products, their formation temperatures and their pyrolysis mechanisms. MA-HCl was placed in a sealed glass tube and heated at the Curie point of a pyrolysis-foil wrapping around the tube. The major pyrolysis processes at temperature below 358°C were N-demethylation and N-methylation reactions of MA. There are sev-

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eral reports about the pyrolytic degradation of MA, while there is no report about a smoking case in which the pyrolysis product is detected in MA abuser's urine.

In a certain smoking case, DMA and its metabolite dimethylamphetamine-N-oxide (DMAO) in addition to MA and amphetamine (AM) were detected in the abuser's urine. But only MA-HCl was detected in the residual colorless crystalline drug that the abuser used; thus, there was a possibility that DMA might be a pyrolysis product formed by heating MA. To clarify whether a pyrolysis product can be detected in the abuser's urine is very important in forensic chemistry, because a pyrolyzate may produce a suspicious result on the identification of the abused drug. We carried out a smoking experiment based on this smoking case, and then gave the case full consideration.

MATERIALS AND METHODS

Materials —— *d*-Methamphetamine HCl (MA-HCl) was purchased from Dainippon Pharmaceutical Co. Ltd., (Osaka, Japan). *d*-Amphetamine $1/2H_2SO_4$ (AM-$1/2H_2SO_4$) and *d*-dimethylamphetamine HCl (DMA-HCl) were synthesized by the method as described previously.[6] All other chemicals used were of the highest purity available commercially. A Supelclean ENVI-Carb SPE cartridge (500 mg) was purchased from Supelco, Inc. (Bellefonte, PA, U.S.A.).

Instrumentation —— GC-MS analyses were carried out on an HP 6890 gas chromatograph (Hewlett Packard Co., Palo Alto, CA, U.S.A.) connected to a JMS-600W mass spectrometer (JEOL Ltd., Tokyo, Japan) using a CP-Sil 8CB column (50 m × 0.32 mm i.d., film thickness 5.0 μm, Chrompak, Middelburg, The Netherlands). The column temperature was raised from 80°C to 230°C at 10°C/min, with a 1 min hold at 80°C and a 9 min hold at 230°C. Helium was used as a carrier gas at a flow rate of 1.5 ml/min. The injection port was used in a splitless mode and maintained at 240°C. Other operating conditions were as follows: electron ionization (EI), electron energy of 70 eV, ion source temperature at 200°C, interface temperature at 240°C, mass range of *m/z* 50 to 500. The quantitative analyses were carried out in selected ion monitoring (SIM) mode using *m/z* 118 for molecular ions (M^+) of Allylbenzene (AB), *cis*-β-methylstyrene (cMS) and *trans*-β-methylstyrene (tMS), *m/z* 106 for M^+ of benzaldehyde (BA), *m/z* 126 for M^+ of benzyl chloride (BC) and *m/z* 120 for M^+ of acetophenone (AP) as internal standard (IS).

For the analysis of a urine sample, liquid chromatography-mass spectrometry in electrospray ionization mode (LC-ESI-MS) was performed using a PU-980 pump (Jasco Corp., Tokyo, Japan) connected to a Platform II mass spectrometer (Micromass Ltd., Manchester, U.K.). Asahipak GS-320HQ (300 mm × 7.6 mm i.d., Showa Denko Ltd., Tokyo, Japan) with a 3 μm pre-filter (5 mm × 4 mm i.d.) and 30% acetonitrile-20 mM ammonium acetate buffer (pH 8.5) were used as an analytical column and mobile phase, respectively. The mobile phase was used at a flow rate of 0.6 ml/min and split so that a flow rate into ESI probe was able to amount to 70 μl/min. The determination of DMA and its metabolites was carried out in the positive ion mode and by monitoring the selected ions as follows: *m/z* 164, 180, 150 and 136, which were protonated molecules $[M+H]^+$ of DMA, DMAO, MA and AM, respectively. The cone voltages were 25 V for DMA, DMAO and MA, and 20 V for AM. The ion source temperature and capillary voltage were kept at 80°C and 3.5 kV, respectively.

For the analyses of the samples except for a urine sample, LC-ESI-MS were performed on an Alliance 2690 high performance liquid chromatograph (Waters Corp., Mass., U.S.A.) connected to a ZMD electrospray ionization mass spectrometer (Micromass Ltd.). At a flow rate of 100 μl/min, 50% of acetonitrile and 20 mM ammonium acetate buffer (pH 5) was passed through a Chiral DRUG column (150 mm × 2 mm i.d., Shiseido Ltd., Tokyo, Japan) with a 3 μm pre-filter (5 mm × 4 mm i.d.). In the previous report,[5] we clarified the formation of *l*-MA, an optical isomer of the starting *d*-MA, by heating at high temperature, and used the chiral column to detect these isomers in this experiment. The ion source temperature, desolvation temperature, capillary voltage and cone voltage were kept at 100°C, 300°C, 3.0 kV and 30 V, respectively. The qualitative and quantitative analyses were carried out in positive ion and full-scan modes in the mass range of *m/z* 50 to 400. The ions used for quantitative analyses were *m/z* 136, 150 and 164, which were $[M+H]^+$ of AM, MA and DMA, respectively.

Calibration Curves —— To prepare calibration standards for GC-MS, the methanol solution containing AB, BA, cMS, BC and tMS was serially diluted with dichloromethane. To 1 ml of the diluted solution containing 0.1, 0.5, 2, 5 and 10 μg/ml of

the five compounds, 50 μl of a methanol solution containing 10.6 μg of AP as IS was added, and 1 μl of the mixed solutions were injected into the GC-MS system. The calibration curves were prepared in correlation with the sample concentrations and their peak area ratios to IS. The calibration curves of AB, BA, cMS, BC and tMS were all linear as follows. AB: $y = -0.0076 + 0.1194X$ [0.1 – 10 μg/ml, r = 0.999], BA: $y = -0.0169 + 0.2177X$ [0.1 – 10 μg/ml, r = 0.999], cMS: $y = -0.0018 + 0.1131X$ [0.1 – 10 μg/ml, r = 0.999], BC: $y = -0.0004 + 0.1165X$ [0.1 – 10 μg/ml, r = 0.999], tMS: $y = -0.0008 + 0.1125X$ [0.1 – 10 μg/ml, r = 0.999].

To prepare calibration standards for the analysis of a urine sample by LC-ESI-MS, the methanol solution containing DMA and its metabolites was serially diluted with acetonitrile. To 300 μl of the diluted solutions, was added 700 μl of 20 mM ammonium acetate buffer (pH 8.5), and each concentration of the mixtures amounted to 0.06, 0.3, 1.5, 2 and 3 μg/ml of DMA, DMAO and MA, and 0.18, 0.9, 4.5, 6 and 9 μg/ml of AM. After the mixtures were stirred, 100 μl of the mixtures were injected into the LC-ESI-MS system, and the calibration curves were prepared in correlation with the sample concentrations and their peak areas. The calibration curves of DMA and its metabolites were all linear as follows. DMA: $y = 7.91 \times 10^4 + 1.96 \times 10^6X$ [0.06 – 3 μg/ml, r = 0.999], DMAO: $y = 1.11 \times 10^5 + 1.23 \times 10^6X$ [0.06 – 3 μg/ml, r = 0.999], MA: $y = 1.61 \times 10^5 + 2.14 \times 10^6X$ [0.06 – 3 μg/ml, r = 0.999], AM: $y = 1.18 \times 10^5 + 8.76 \times 10^5X$ [0.18 – 9 μg/ml, r = 0.999].

For LC-ESI-MS analyses, except for a urine sample, the methanol solution containing AM, MA and DMA was serially diluted with 20 mM ammonium acetate buffer (pH 5). After the mixtures were stirred, 10 μl of the calibration samples containing 25, 50, 100, 250 and 500 ng/ml of the three compounds were injected into the LC-ESI-MS system, and the calibration curves were prepared in correlation with the sample concentrations and their peak areas. The calibration curves of AM, MA and DMA were all linear as follows. AM: $y = -170 + 59.16X$ [25 – 500 ng/ml, r = 0.999], MA: $y = -787 + 175.66X$ [25 – 500 ng/ml, r = 0.999], DMA: $y = -1328 + 139.52X$ [25 – 500 ng/ml, r = 0.999].

Profiles of Smoking Case ——— A drug abuser, a 28-year-old male, put a colorless crystalline drug in a glass tube, 50 mm length and 5.7 mm i.d., and heated it with a gas lighter for inhaling the released vapors through a straw. A urine sample was collected after four hours from the intake, and its pH was 5.3. The used glass tube and 145 mg of the residual colorless crystalline drug were confiscated. Some humid colorless crystals, humid yellow solid materials and scorches were found to adhere on the inside of the glass tube, and the total amount was 14 mg. The drug abuser testified that he smoked the same colorless crystalline drug four times at intervals of several days without washing the same glass tube.

Analytical Procedures in Smoking Case ——— The urine sample was analyzed by the same method as in the author's previous paper.[7] To 700 μl of abuser's urine or appropriately diluted urine with distilled water, were added 300 μl of acetonitrile and 30 μl of 2.8% aq. ammonia, and the mixture was stirred with a vortex mixer. After the mixture was centrifuged at 2000 $\times$ g for 5 min, the supernatant was passed through a 0.5 μm membrane filter, and 100 μl of eluate was injected into the LC-ESI-MS system.

The residual materials inside the glass tube used for the smoking were rinsed with a small amount of methanol. The colorless crystalline drug that the drug abuser used was dissolved in 5 ml of methanol. Both methanol solutions were appropriately diluted with 20 mM ammonium acetate buffer (pH 5), and 10 μl of the diluted solutions were analyzed using the LC-ESI-MS system.

Smoking Experiments ——— Figure 1(A) depicts the smoke collection apparatus used in this smoking experiment, which is identical with that used in the previous report.[8] The crystals of MA-HCl were heated with a gas lighter, and the generating vapor was collected on a solid phase extraction (SPE) cartridge. A thermocouple was placed on the bottom of a glass tube, and the variations in temperature at the bottom of the glass tube were recorded through the thermocouple by using a DC3100 Remote Scanner Jr. (NEC-Sanei, Tokyo, Japan). The procedures of heating MA-HCl and collecting the vapor followed the schematic diagram in Fig. 1(B). On the bottom of the glass tube, 10 mg of MA-HCl was placed, and heated with a gas lighter for 30 sec, then for 10 sec at 10 sec intervals two times. The first collection using a 35 ml puff for 2 sec was carried out at the end of heating for 30 sec, and then three collections were performed at 15 sec intervals. The vaporizing temperature of *d*-MA-HCl was 218°C by measuring with thermogravimetry, and the major pyrolysis process at temperature below 358°C was N-demethylation and N-methylation reactions of MA, which yielded AM and DMA, respectively.[5] Thus, the temperature at the bottom of the glass tube

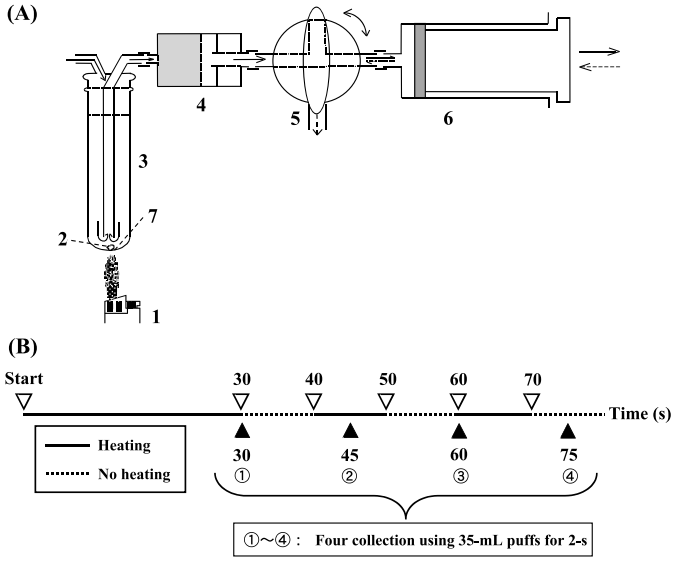

Fig. 1. Schematic Diagram of Smoke Collection Apparatus (A) and Smoke Collecting Procedure (B)
1; Gas lighter, 2; MA-HCl (10 mg), 3; Glass tube (10.5 cm, 1.5 cm i.d.), 4; SPE cartridge, 5; Three-way cock, 6; 50 ml-Syringe, 7; The position where temperature variations were measured with a thermocouple.

was kept below 350°C and adjusted to the range from 250 to 350°C during the four smoke collections. No drug was visibly present at the bottom of the glass tube after the last collection.

After the smoke collection apparatus has been cooled to room temperature, the pyrolysis products were desorbed from the SPE cartridge with 6 ml of methanol. The residual materials inside the smoke collection apparatus were rinsed with 2 ml of methanol. For qualitative analyses, 1 μl of both methanol solutions were injected into the GC-MS system. For quantitative analyses, the methanol solutions of the cartridge eluate and residual materials were both diluted to a fifth with dichloromethane. To 1 ml of each diluted solution, was added 50 μl of the methanol solution containing 10.6 μg of AP as IS, and 1 μl of the mixture was injected into the GC-MS system. The methanol solution of the cartridge eluate was diluted with 20 mM ammonium acetate buffer (pH 5) to one two-thousandth or one two-hundredth, while the methanol solution of the residual materials was diluted to one ten-thousandth or one thousandth. Each diluted solution was passed through a 0.45 μm membrane filter, and 10 μl of the eluate was injected into the LC-ESI-MS system.

RESULTS

Analyses of Smoking Case

Figure 2 shows SIM chromatograms obtained from the abuser's urine by LC-ESI-MS. In the abuser's urine, 38.4 μg/ml of MA, 4.94 μg/ml of AM, 0.44 μg/ml of DMA and 0.89 μg/ml of DMAO were detected. Only *d*-MA was detected in the colorless crystalline drug confiscated from the drug abuser. Figure 3(A) shows a total ion chromatogram (TIC) and extracted ion chromatograms of the residual materials inside the glass tube by LC-ESI-MS. In addition to *d*-MA, *d*-AM and *d*-DMA were detected in the residual materials, and the formation ratios of *d*-MA, *d*-AM and *d*-DMA to the total of three compounds were 82.7, 11.5 and 5.8%, respectively.

Pyrolysis Products in Smoking Experiments

Figure 4 depicts a typical TIC and extracted ion chromatograms of the cartridge eluate by GC-MS. The peaks at retention time (RT) 13.9, 14.3, 14.8, 15.4 and 15.5 min were identified as AB, BA, cMS, BC and tMS, whose M^+ were *m/z* 118, 106, 118, 126 and 118, respectively, by comparison with RTs and mass spectra of authentic chemicals. The peaks at RT 17.5 and 20.2 were AM and DMA, whose base peaks were *m/z* 44 and 72, respectively, by direct comparison with authentic chemicals. Figure 3(B) shows a typical TIC and extracted ion chromatograms of the cartridge eluate by LC-ESI-MS. The

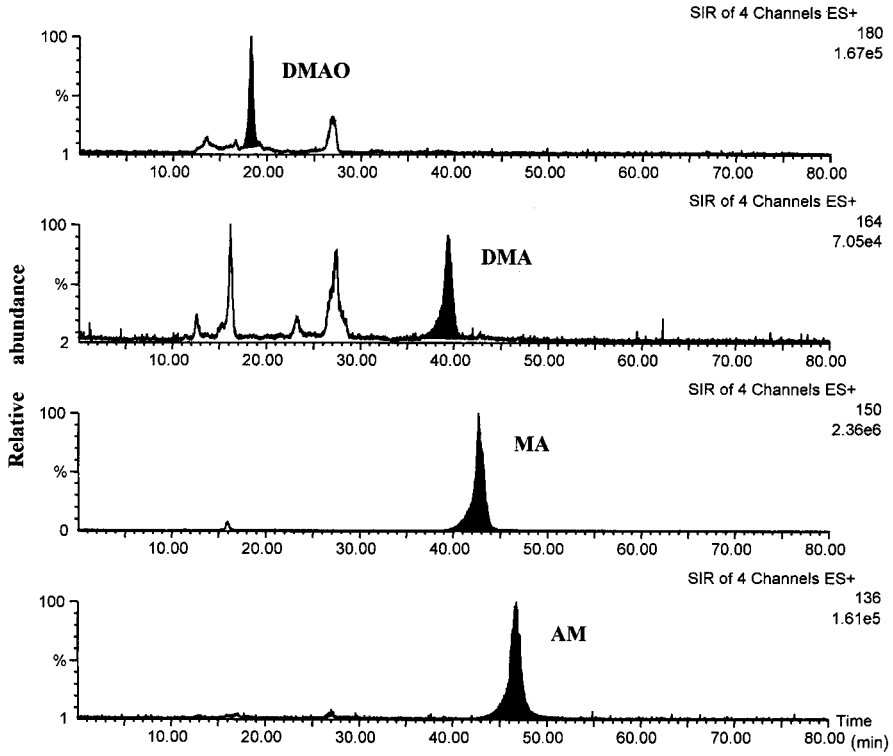

Fig. 2. SIM Chromatograms of Abuser's Urine by LC-ESI-MS

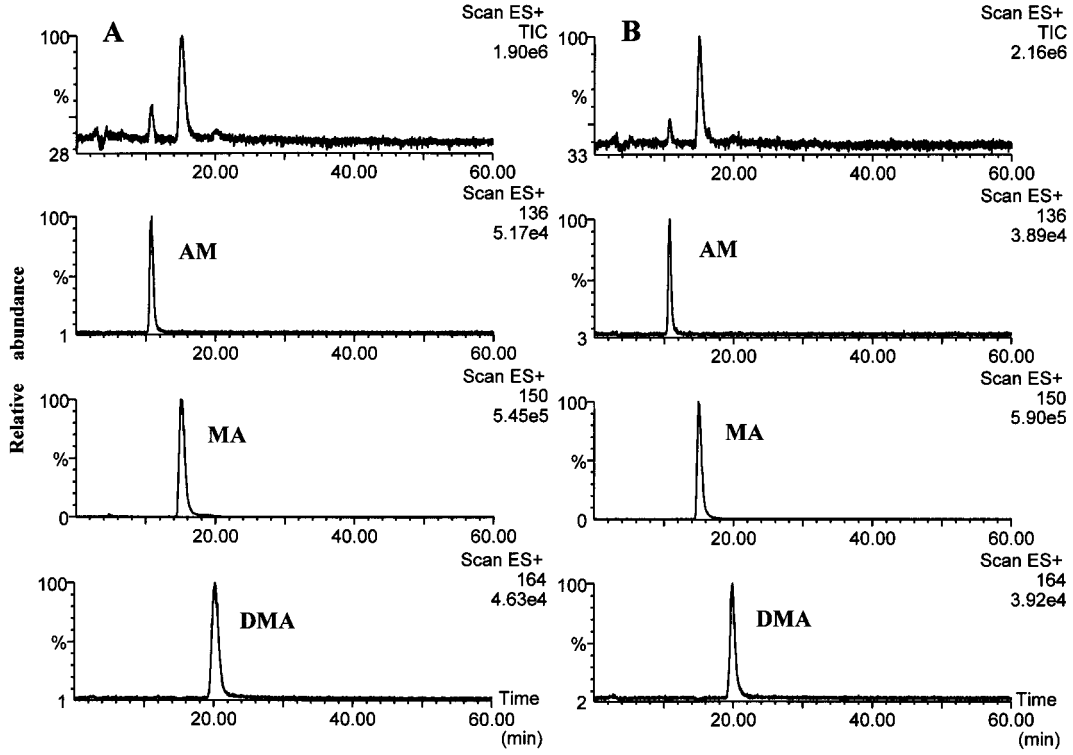

Fig. 3. Total Ion Chromatograms and Extracted Ion Chromatograms of Residual Materials in Smoking Case (A) and Cartridge Eluate in Smoking Experiment (B) by LC-ESI-MS

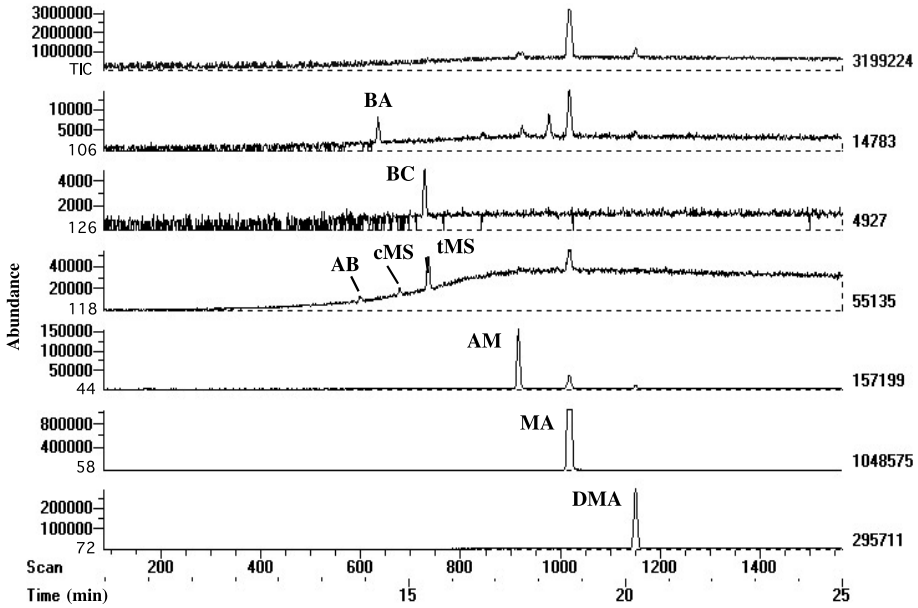

Fig. 4. Typical Total Ion Chromatogram and Extracted Ion Chromatograms of Cartridge Eluate by GC-MS

Table 1. Formation Ratios of MA Pyrolysis Products

	Cartridge eluate	Residual materials in smoke collection apparatus	Total
Compound	Formation ratio (%)[a)]	Formation ratio (%)	Formation ratio (%)
AM	2.59 ± 1.12	6.93 ± 1.72	9.52
MA	21.8 ± 9.7	63.9 ± 7.2	85.7
DMA	1.88 ± 0.19	3.10 ± 1.33	4.98
AB	0.06 ± 0.02	N.D.[b)]	0.06
BA	0.06 ± 0.01	0.03 ± 0.01	0.09
cMS	0.09 ± 0.03	N.D.	0.09
BC	0.07 ± 0.06	0.03 ± 0.02	0.10
tMS	0.45 ± 0.20	0.02 ± 0.01	0.47
Total	27.0	74.0	101.0

a) The values are the molar percentages of each pyrolysis product in relation to the starting MA. Each value is expressed as the mean ± S.D., $n = 3$. *b*) N.D. = not detected.

peaks at RT 10.8 and 19.9 min were confirmed as *d*-AM and *d*-DMA, whose $[M+H]^+$ were *m/z* 136 and 164, respectively, by direct comparison with authentic chemicals. In the previous report,[5)] we found that the optical isomer of the starting *d*-MA, or *l*-isomer of MA, and *l*-isomers of AM and DMA, were formed at 445°C simultaneously. *l*-Amphetamines were not formed in this smoking experiment, which was carried out at a maximum temperature of 350°C.

Table 1 shows the formation ratio of each pyrolysis product to the starting MA. A large portion of a pyrolyzate AM and DMA, and a non-pyrolyzate MA were deposited on the cool wall inside the smoke collection apparatus after being vaporized. The sum of formation ratio of DMA in the cartridge eluate and that in the residual materials was 4.98%. The adsorption of the pyrolyzates containing DMA on the cartridge means that these pyrolyzates can be taken into an abuser's body. The ratio of DMA to AM in this experiment was 52.3%.

DISCUSSION

DMA and its metabolite DMAO in addition to MA and AM were detected in smoking abuser's urine. The excreted amounts of DMA and DMAO were both below 3% of that of MA and remarkably

low. But only MA-HCl was detected in the residual colorless crystalline drug that the abuser used. In the smoking experiment, the formation ratio of DMA amounted to about 5% under the experimental conditions used, thus there was a possibility that DMA might be a pyrolysis product formed by heating MA.

The total formation ratios of AM and DMA to the starting MA in the smoking experiment were 9.52% and 4.98%, respectively. The ratio of DMA yield to AM yield was 52.3%, and the formation ratio of DMA was nearly a half of that of AM under the experimental conditions used. In the previous reports,[5,6] we indicated that only a methyl cation eliminated from a N-methyl group participated in the N-methylation, or the formation of DMA, moreover the reactions of the eliminated methyl cation with chlorine anion and trimethylamine afforded methyl chloride and tetramethylammonium, respectively. Accordingly, we can presume that N-methylated product DMA will have a lower formation ratio than N-demethylated product AM. In other words, it may be inferred that a given smoking abuser will use MA containing DMA if the formation ratio of DMA is equal to that of AM, or more than that of AM in the residual materials adhering on a smoking apparatus. In the smoking case, the formation ratios of AM and DMA inside the glass tube used were 11.5 and 5.8%, respectively. The ratio of DMA yield to AM yield in the smoking case was 50.4%, and was close to that in the smoking experiment.

The vaporizing temperature of *d*-MA-HCl was 218°C by measuring with thermogravimetry. In the previous report,[5] the demethylation and methylation reactions of MA were identified to occur at 315°C. In the smoking case, some scorches were found inside the glass tube used for heating. Therefore, we predicted that MA was heated at relatively high temperature. Moreover, the enhancement of the formation ratios of AM and DMA may be caused by the smoking means, that is, repeated use of the same glass tube four times. There will be no inconsistencies in considering that AM and DMA have been formed as pyrolysis products of MA in the smoking case, and DMA and its metabolite DMAO have been detected in the urine of this smoking abuser.

Most smoking abusers heat the drug on a piece of aluminum foil. On the aluminum foil, the drug is easily vaporized at temperature above vaporizing temperature 218°C, and hence it is difficult for the drug to be kept at the higher temperature. This smoking experiment was carried out in a limited space and at the temperature range, which are apt to cause the demethylation and methylation. Therefore, the experimental formation ratios of demethylated and methylated products may get higher than those in the smoking case using a piece of aluminum foil. The formation ratio of DMA in this experiment is supposed to be approximate to the maximum in real smoking cases. It does not necessarily follow that smoking MA produces DMA as a pyrolysis product; or rather it seems likely that this smoking case is rare. This examination of the smoking case, which may discover the cause of the discrepancy induced by smoking, is very valuable in forensic chemistry.

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