Studies on 1-(2-Phenethyl)-4-(*N*-Propionylanilino)Piperidine (Fentanyl) and Its Related Compounds: Novel Metabolites in Rat Urine Following Injection of α-Methylfentanyl, One of the Most Abused Typical Designer Drugs

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Although 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) is controlled by drug control laws, its slightly modified compounds, which show the same analgesic activities, cannot be controlled legally. Among these fentanyl analogues, 1-[2-(2-methylphenethyl)]-4-(*N*-propionylanilino)piperidine (α-methylfentanyl) is the typical and most widely abused drug and its overdose has caused a number of fatalities. Analysis of the urine of addicts has been widely performed to detect its metabolites and the unchanged compound for proof of its abuse. In this case, the metabolites detected in urine should reflect the structure of the original compound. In the present report, for clarification of α-methylfentanyl abuse, four novel metabolites, which reflect the original structure of α-methylfentanyl, were identified in rat urine. One of these was the *p*-hydroxy form of the aromatic ring of the α-methylfentanyl phenethyl group (mono-aromatic hydroxy α-methylfentanyl), while the second and third ones were metabolites of ω-1 or ω position hydroxypropionyl of α-methylfentanyl (mono-hydroxypropionyl α-methylfentanyl). The fourth one was a metabolite involving the *p*-hydroxy form of the aromatic ring of the phenethyl group and ω position of hydroxypropionyl α-methylfentanyl (di-hydroxy α-methylfentanyl). The structures of these compounds were identified by comparisons of their retention times and mass spectra obtained by gas chromatography-mass spectrometry (GC/MS) and mass chromatography of mono- and di-hydroxy α-methylfentanyl with those of the synthesized authentic compounds.

Key words —— forensic, α-methylfentanyl, designer drug, metabolite, GC/MS

INTRODUCTION

Due to its strong analgesic activity, 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) is widely utilized as a medicine for the terminal phase of cancer patients in order to remove pain. However, because of its strong analgesic activity, drug abuse of its structurally modified compounds, so-called "designer drugs," has occurred. Although these drugs were slightly changed the structure of fentanyl, they have similar or greater analgesic and euphoric activities. Furthermore, these drugs were not able to prohibited by the old drug control laws, and their high toxicity caused several fetal cases. In the first report, these drugs were called bogus "China White." The term of "China White" was a slang of heroin.[1] But now many fentnayl analogues are called "China White" such as 3-position of the piperidine ring was methylated fentanyl, it was described as 3-methylfentanyl,[2–6] and α-position of phenethyl group was methylated one was also called α-methylfentanyl. In a previous study, the reported mass spectrum of bogus "China White" was compared with those of synthesized fentanyls, and reported structure of bogus "China White" was presumed to involve the introduction of a methyl group to the α-position of the phenethyl group of fentanyl (α-methylfentanyl).[7] Furthermore, several discrimination methods for fentanyl and its analogues have been reported.[8–13] The structures of fentanyl, 3-methylfentanyl and α-methylfentanyl are shown in Fig. 1.

Regarding the drug control laws in Japan, the

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possession, illegal import and transfer of abused drugs were prohibited, furthermore, the use of narcotics, stimulants and other drugs with defined structures, are also strictly prohibited. For this reason, examination of urine is widely performed in forensic laboratories in order to prove their usage. As an example, the major abused drug in Japan is methamphetamine, and to prove its usage, about 20000 urine samples were positive in 2006 of the urine samples examined for the detection of unchanged methamphetamine and its major metabolite, amphetamine.[14] This number of examinations is far higher than the number of methamphetamine crystal cases. Similar to the case for methamphetamine, analysis of urine or blood should be performed to detect fentanyl metabolites that have the same structure as the original abused drug. There are many published papers regarding the metabolism of fentanyl itself,[11–13, 15–18] and recent enzymatic studies of fentanyl have involved human hepatocytes and P-450 3A4.[19, 20] However, none of these reports except one, ω position hydroxypropionylfentanyl (ω hydroxyfentanyl), have not focused on metabolites that reflect the structure of the dosed compound.[11–13] The metabolites of fentanyl are shown in Fig. 2.

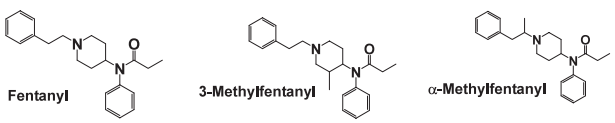

Fig. 1. Structures of Fentanyl, 3-Methylfentanyl and α-Methylfentanyl

Although there are a quite few reports of the metabolism of fentanyl analogues,[21] there are no reports regarding the metabolism of α-methylfentanyl, the major abused fentanyl analogue, even though it is presently controlled as a narcotic by the drug control laws. Furthermore, there are no reports on metabolites with structures that reflect the structure of the dosed α-methylfentanyl in order to define its use. In the present report, four novel metabolites with structures that reflect the dosed drug, α-methylfentanyl, were identified in rat urine. The existence of characteristic metabolites of α-methylfentanyl was presumed by the observed peaks on a total ion chromatogram (TIC) of trifluoroacethylated rat urine extract and the mass spectra of their trifluoroacetyl derivatives. Their structures were confirmed by comparisons of the retention times and mass spectra of trifluoroacetyl

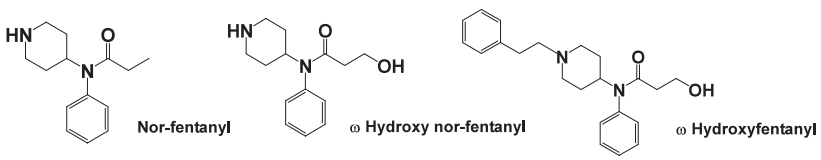

Fig. 2. Structures of Major Metabolites of Fentanyl, Nor-fentanyl, ω-Hydroxy nor-fentanyl and ω-Hydroxyfentanyl

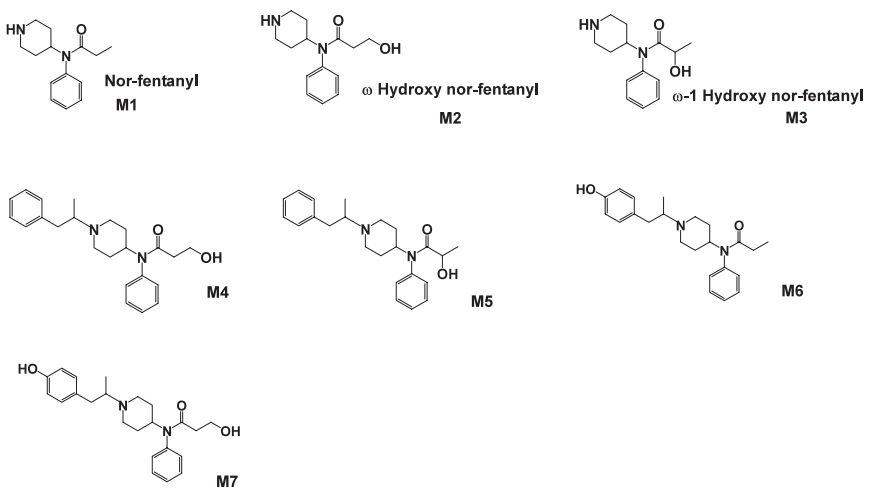

Fig. 3. Presumed Metabolites of α-Methylfentanyl (M1 to M7) of Rat
M1 and M2 are same metabolites found in fentanyl administrated rat urine.

derivatives obtained by gas chromatography-mass spectrometry (GC/MS) with those of the authentic chemically synthesized compounds. The structures of metabolites (M1 to M7) of α-methylfentanyl in rat urine are summarized in Fig. 3.

MATERIALS AND METHODS

Chemicals —— Fentanyl was purchased from Sankyo Co. Ltd. (Tokyo, Japan). The reagents for the synthesis of its metabolites were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The compounds 1-carbetoxy-4-piperidone and 1-(p−benzoxyphenyl)-2-propanone were obtained from Aldrich Co. Inc. (St. Louis, MO, U.S.A.). β-Glucuronidase used for enzymatic digestion of the glucuronized metabolites was obtained from Sigma Co. Inc. (Milwaukee, WI, U.S.A.). α-Methylfentanyl was prepared according to a previous report.[7] Other chemicals were of analytical grade and the water used was prepared with a Milli-Q system (Nihon Millipore Inc., Tokyo, Japan).

Animal and Urine Samples —— Male Wistar rats (120–150 g) were used in this study. A total of 10 rats were placed in metabolic cages separately for urine collection. α-Methylfentanyl was injected periorally at a dosage of 3 mg/kg in a day for 5 days. This dosage level was considered to represent the LD_{50} of each rat before examination. Urine was collected every 24 hr for each rat. The samples were combined and stored at −10°C in the freezer until analysis.

Extraction of Metabolites from Urine —— A 50 ml portion of the collected urine was adjusted to pH 5.0 with 1 M acetic acid (approximately 5 ml added). Next, 500 U/ml urine of β-glucuronidase was added and the mixture was incubated at 37°C for 24 hr. After the incubation, the pH of the mixture was adjusted to 8.5 with 1 M sodium carbonate (approximately 10 ml added) and the metabolites of α-methylfentanyl excreted into the urine were extracted three times with 50 ml of chloroform–isopropanol (3:1, v/v). The extracts were combined and the solvent was removed at 30°C in a water bath by an evaporator.

Trifluoroacetylation of Rat Urine Extracts —— The extract was dissolved in 2 ml of ethylacetate and placed in a reaction vial, followed by removal of the ethylacetate under N_2 flow in a heating block at 30°C. The residue was re-dissolved in 100 μl of ethylacetate, and 50 μl of trifluoroacetic acid anhydrate was added to 100 μl of the extract solution. The reaction vial was sealed and placed in an aluminum-heating block at 40°C for 30 min. After removal of the ethylacetate and trifluoroacetic acid under N_2 flow in the heating block at 30°C, the residue was re-dissolved in 50 μl of ethylacetate, and 5 μl of this solution was injected into GC/MS.

GC/MS and Mass Chromatography Conditions —— GC/MS was conducted by the following condition. GC/MS instrument was M-80 sector type gas chromatograph/mass spectrometer supplied by Hitachi, Co. Ltd. (Tokyo, Japan). GC column: 1% OV-17/Chromosorb W (AW-DMCS); mesh, 100–120; length, 2 m; diameter, 3 mm. Column temperature: 130–270°C (5°C/min) and hold at 270°C for 20 min, injection temperature: 260°C, interface temperature: 260°C and carrier gas: N_2, 50 ml/min. Ionization energy: 100 eV, ionization current: 110 μA, ionization method: chemical ionization (CI) mode, reaction gas: iso-butane.

To obtain their full mass spectra, scan range was from m/z 80–700 and for the mass chromatography, m/z 463 and 259 for mono-hydroxy α-methylfentanyl and m/z 575 and 371 for di-hydroxy α-methylfentany were selected. The obtained mass chromatogram is shown in Fig. 4.

Synthesis of Presumed Metabolites in Rat Urine —— M1 (nor-fentanyl), M2 (ω hydroxy nor-fentanyl) and M3 (ω-1 hydroxy nor-fentanyl) were synthesized from 1-carbetoxy-4-piperidone (1) as a starting compound. Through 1-carbetoxy-4-anilino-piperidine (2), 4-anilinopiperidine (3), 1-carbobenzoxy-4-anilinopiperidine (4), 1-carbobenzoxy-4-propionylanilinopiperidine (5), finally 4-propionylanilino-piperidine (nor-fentanyl, M1, 6) was produced (Fig. 5a). For the synthesis of M2, in order to synthesize the ω hydroxypropionyl group instead of the propionyl group, started by the formation of the mono potassium salt (8) of diethylmalonate (7), by its reduction to form monoethylmalonate (9), finally monoethylmalonic chloride (10) was obtained. This compound (10) was attached to 4, to yield 1-carbobenzoxy-4-(anilino ω ethylmalonate)-piperidine (11). Following reduction of ω position of 11, 1-carbobenzoxy-4-(ω hydroxypropionylanilino)-piperidine (12) was obtained, and removal of carbobenzoxy group of 11, 4-(ω hydroxylpropionylanilino)-piperidine (ω hydroxy nor-fentanyl, M2, 13) was produced (Fig. 5b). The synthesis of M3 was similar to the synthesis of M2. The start-

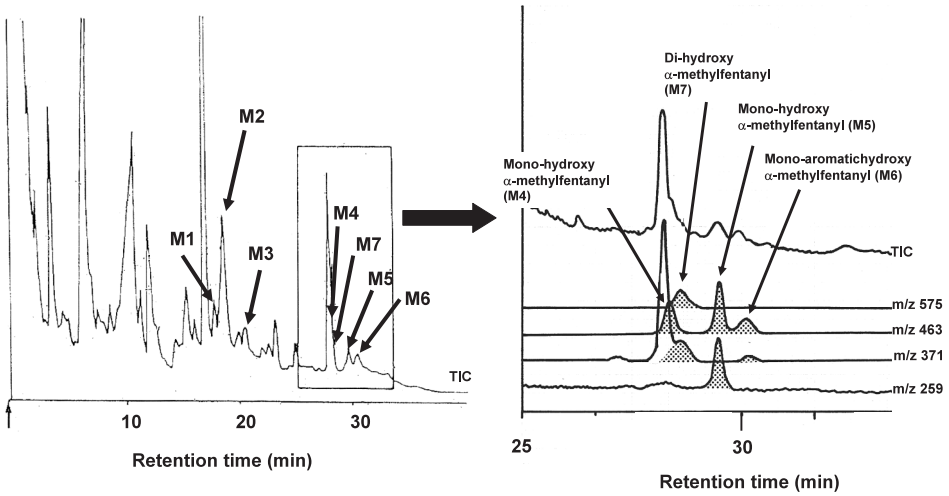

Fig. 4. TIC and Mass Chromatogram

m/*z* 463 and 259 for mono-hydroxy α-methylfentanyl (M4, M5 and M6) and *m*/*z* 575 and 371 for di-hydroxy α-methylfentanyl (M7) of trifluoroacetyl derivatives in rat urine extract.

ing compound was pyruvylchloride (15) obtained from pyruvic acid (14) and formylchloride, through 1-carbobenzoxy-4-(pyruvicanilino)-piperidine (16) produce 1-carbobenzoxy-4-(ω-1 hydroxypropionylanilino)-piperidine (17). After removal of the 1-carbobezoxy group in the same way as for the synthesis of M2, 4-(ω-1 hydroxypropionylanilino)-piperidine (ω-1 hydroxy nor-fentanyl, M3, 18) was obtained (Fig. 5c). The synthetic pathways of M1, M2 and M3 are summarized in Fig. 5a, 5b and 5c.

For the synthesis of M4, 1-phenyl-2-bromopropane was reacted with (M2, 13) and ω hydroxy α-methylfentanyl (M4, 19) was obtained. The mass spectrum of trifluoroacetyl derivative of M5 (20) was exactly the same as that of M4. It was considered that this compound was ω-1 hydroxy α-methylfentanyl (20). The synthesis of M5 was started by combining M3 (18) and 1-phenyl-2-bromopropane, following, ω-1 hydroxypropionyl α-methylfentanyl (M5, 20) was obtained.

In the metabolism of aromatic hydroxylation, most cases should occur at the *p*-position of the aromatic ring. Metabolite M6 was considered to be *p*-hydroxyl α-methylfentanyl. For the above reason, nor-fentanyl (M1, 6) was combined with 1-(3-carbobenzoxy)-2-bromo-propane (21), and following removal of carbobenzoxy group yielded *p*-hydroxyl α-methylfentanyl (M6, 23). The synthetic schemas of M4, M5 and M6 are summarized in Fig. 6.

Metabolite M7 was considered to be di-hydroxy α-methylfentanyl. One of the hydroxy positions was presumed to be at the *p*-position of the aromatic ring, but the other position was not defined as the ω or ω-1 position of the propionyl group by the mass spectrum. For the above reason, both di-hydroxy α-methylfentanyls were synthesized. *p*-Aromatic and ω hydroxy α-methylfentanyl (M7, 24) was synthesized by combining ω hydroxy nor-fentanyl (M2, 13) and 21, followed by reduction. The metabolite of *p*-aromatic hydroxy and ω-1 hydroxyl α-methylfentanyl (25) was also obtained by the same method utilizing ω-1 hydroxy nor-fentanyl (M3, 18) and 21. Both trifluoroacetylated compounds were injected into the GC/MS. The retention time of 24 was the same as that of the metabolite found in rat urine. From this result, metabolite M7 was determined to be *p*-aromatic and ω hydroxy α-methylfentanyl (24). The synthetic pathways of M7 and *p*-aromatic and ω-1 hydroxy α-methylfentanyl (**25**) are summarized in Fig. 7.

RESULTS AND DISCUSSION

GC/MS and Mass Chromatography for Detection of Metabolites with Structures that Reflect α-Methylfentanyl

In all previous reports regarding the metabolism of fentanyl,[11–13, 15–18] the major metabolite was nor-fentanyl (M1), furthermore, other metabolites of ω position hydroxypropionyl nor-fentanyl (ω hydroxy nor-fentanyl, M2) and ω hydroxyfentanyl were also reported.[11–13] With the exception of

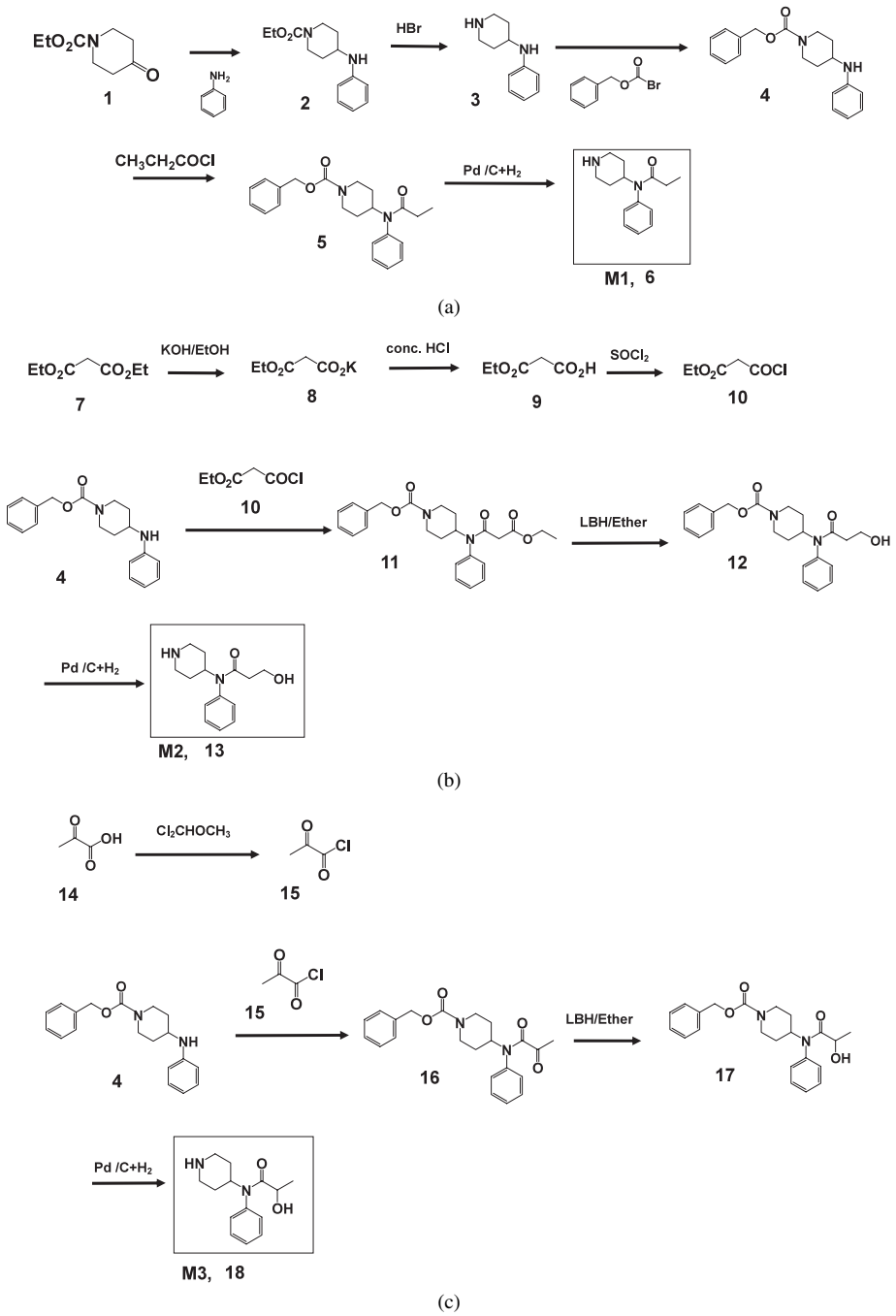

Fig. 5. a: Synthetic Schemas of Nor-Fentanyl (M1), b: ω-Hydroxy Nor-Fentanyl (M2) and c: ω-1 Hydroxy Nor-Fentanyl (M3)

ω hydroxyfentanyl, metabolites that preserved the original structure of fentanyl were not reported. The structures of major metabolites, such as, nor-fentanyl and ω hydroxy nor-fentanyl are also summarized in Fig. 2 with ω hydroxyfentanyl. Metabolites M1and M2 could be found in both fentanyl and α-methylfentanyl injected cases, but M3 is specific metabolites in α-methylfentanyl case, and ω hydroxyfentanyl is also specific metabolite in fentanyl case.

Although there are no reports on the metabolism of α-methylfentanyl itself, its metabolites are considered to have similar characters to those of fentanyl. The assignment of these metabolites was conducted by the comparisons with authentic chemically synthesized compounds.

The major metabolite was the same to the major metabolite of fentanyl, and involved the removal of a 1-pheny-2-methylethyl group attached to the 1-position of the piperidine ring of α-methylfentanyl

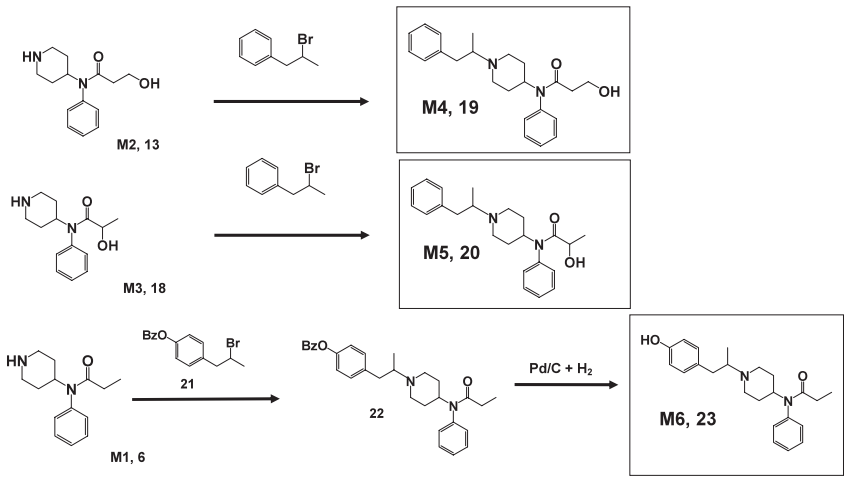

Fig. 6. Synthetic Schemas of ω Hydroxy α-Methylfentanyl (M4), ω-1 Hydroxy α-Methylfentanyl (M5) and p-Aromatic Hydroxy α-Methylfentanyl (M6)

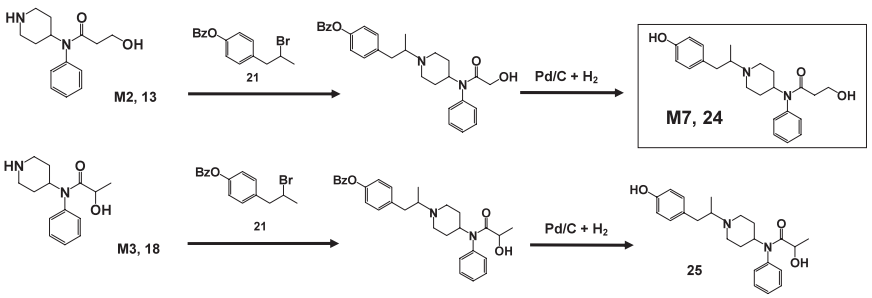

Fig. 7. Synthetic Schemas of p-Aromatic Hydroxy and ω Hydroxy α-Methylfentanyl (M7), and p-Aromatic Hydroxy and ω-1 Hydroxy α-Methylfentanyl

(nor-fentanyl, M1), and the same metabolites as fentanyl were defined as mono-hydroxy metabolites of nor-fentanyl, such as ω hydroxy nor-fentanyl (M2). In addition to these metabolites, another mono-hydroxy metabolite of α-methylfentanyl, which showed the same spectrum as M2, was observed on mass chromatogram (M3). The structure of M3 was presumed to be ω-1 hydroxy nor-fentanyl. The presumed structures of the trifluoroacetyl derivatives and their CI mass spectra of compounds M1, M2 and M3 are shown in Fig. 8.

These metabolites did not reflect the structure of the dosed drug, α-methylfentanyl. Metabolites, M1 and M2 were the same metabolites as those of fentanyl. In order to determine metabolites that reflected the structure of the dosed drug, α-methylfentanyl, mass chromatography utilizing the mass numbers of trifluoroacetylated mono- and di-hydroxy α-methylfentanyl (m/z 463 and 259 for mono-hydroxy α-methylfentanyl and m/z 575 and 371 for di-hydroxy α-methylfentanyl, respectively) was conducted. The TIC and results of mass chromatography at m/z 463 and 259 and m/z 575 and 371 are also shown in Fig. 4. On the mass chromatogram, in addition to M1, M2 and M3, three mono-hydroxy α-methyl fentanyl peaks of possible candidates, comprising three metabolites of mono-hydroxy α-methylfentanyl (M4, M5 and M6) and one metabolite of di-hydroxy α-methylfentanyl (M7) were observed. The mass spectra of trifluoroacetyl derivatives of M4, M5, M6 and M7 are shown in Fig. 8. In addition to the mono-hydroxyl α-methylfentanyl, the trifluoroacetyl derivative of di-hydroxy α-methylfentanyl is also shown in Fig. 8. The metabolites names of M1 to M7 are correspond to the each observed peak on the mass chromatogram shown in Fig. 4.

The Novel Metabolites Identified Its Structure in Rat Urine

From the detail observation of TIC and mass chromatogram of extract of α-methylfentanyl in-

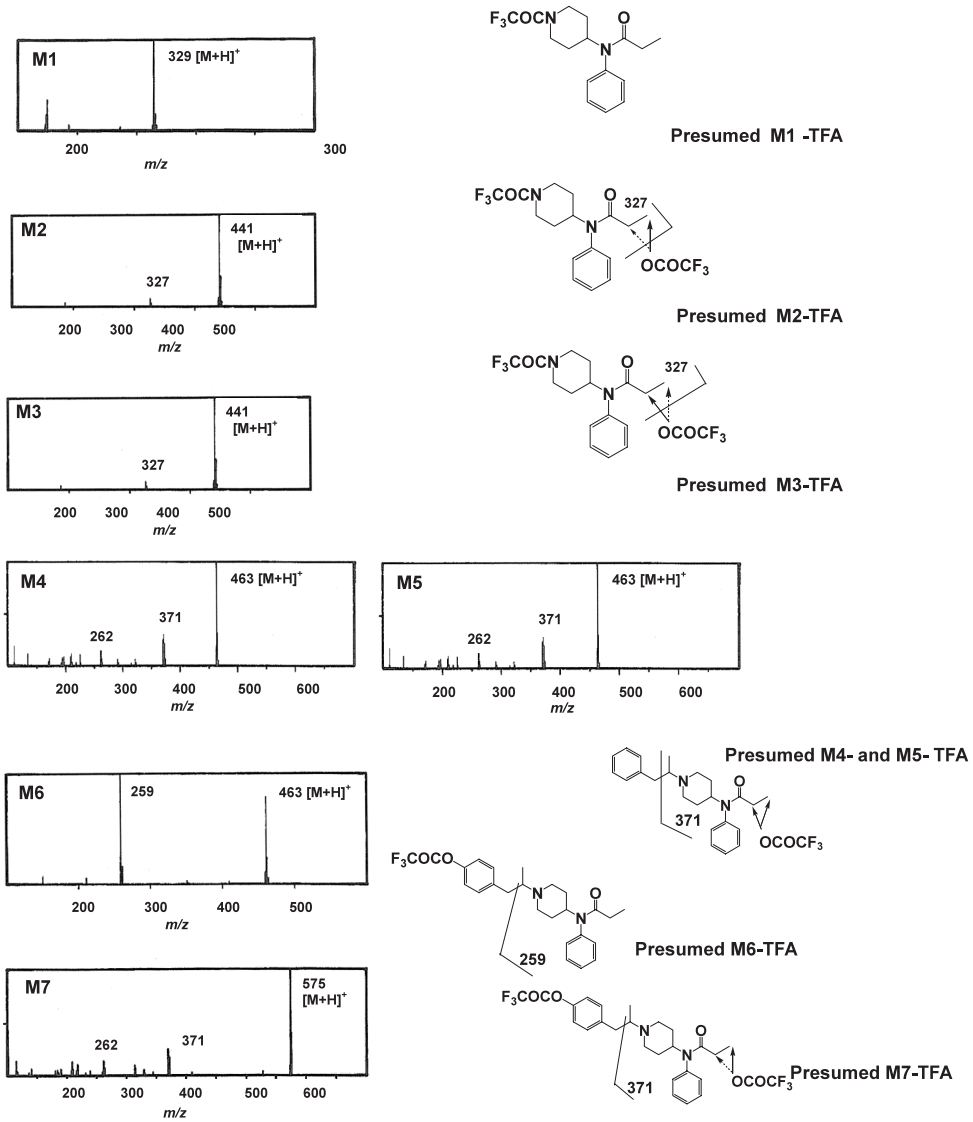

Fig. 8. Mass Spectra of Trifluoroacetyl Derivatives that Retain the Structure of α-Methylfentanyl

nor-fentanyl (M1), ω-, ω-1 hydroxy nor-fentanyl (M2 and M3), ω-, ω-1 hydroxy α-methylfentanyl (M4 and M5), aromatic hydroxy α-methylfentanyl (M6) and aromatic hydroxy and ω- or ω-1 hydroxy α-methylfentanyl (M7).

jected rat urine, 4 novel metabolites that reflect the structure of dosed drug, α-methylfentanyl were found. Their structures were presumed to be M4, M5, M6 and M7 shown in Fig. 8. For the identification of their structure, presumed metabolites were synthesized.

In the case of M4 and M5, these two compounds showed identical mass spectra and hydroxy positions were presumed to be ω and ω-1 position of propionyl group. The synthetic pathways of these two metabolites are shown in Fig. 6. The retention times and mass spectra of trifluoroacetylated M4 and M5 were same to presumed M4 and M5 in Fig. 8. Presumed M6 in Fig. 7 was aromatic ring of phenethyl group hydroxy metabolite. To confirm hydroxy position at aromatic ring, *p*-hydroxy α-methylfentanyl (M6) was synthesized. The detail synthetic method is shown in Fig. 6. The identity of presumed M6 and M6 was proved in the same manner to M4 and M5. Presumed M7 in Fig. 8 was considered di-hydroxy α-methylfentanyl, and hydroxy positions were aromatic ring and propionyl group from the mass spectrum of trifluoroacetyl derivative of presumed M7. As a result, M7 was ω hydroxyl, not ω-1 hydroxyl propionyl group and also *p*-aromatic hydroxy metabolite. The synthetic method of M7 is shown in Fig. 8.

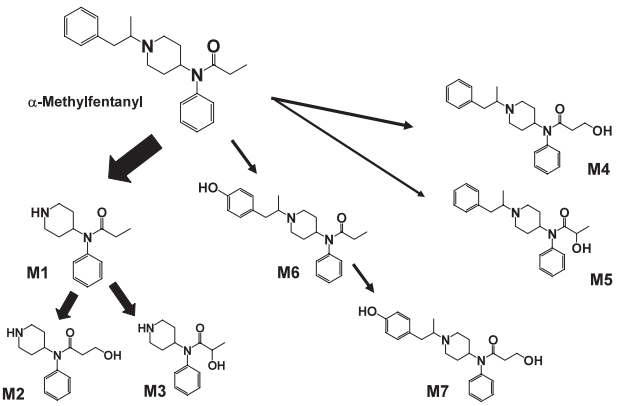

Fig. 9. Expected Metabolic Pathway of α-Methylfentanyl in Rat

Expected Metabolic Pathway of α-Methylfentanyl

Based on the results obtained in the present study, the expected metabolic pathways of α-methylfentanyl in rat are shown in Fig. 9.

Main pathway of metabolism of α-methylfentanyl was shown by bold arrows, it was identical to that of fentanyl, the main metabolites found in urine was nor-fentanyl and ω hydroxy nor-fentanyl. But to specify the intake of α-methylfentanyl, four novel metabolites were found in rat urine by mass chromatography. These compounds were three of mono-hydroxy α-methylfentanyl and one of di-hydroxy α-methylfentanyl. By the synthesis of authentic compounds of these four metabolites, two of mono-hydroxy α-methylfentanyls were ω and ω-1 hydroxy α-methylfentanyl, and other mono-hydroxy α-methylfentanyl was p-position of aromatic ring of phenethyl group hydroxy α-methylfentanyl. One of di-hydroxy α-methylfentanyl was identified as ω position of propionyl group and p-position of aromatic ring of phenethyl group was also hydroxy α-methylfentanyl. From the major metabolites in rat urine, the intake compound taken could not be specified, but these four metabolites were reflect the intake compound, α-methylfentanyl and from these metabolites excreted in rat urine, intake compounds could be specified.

In conclusion, structurally modified compounds of legally prohibited drugs by drug control laws (designer drugs) have been widely abused, and fentanyl analogues were the first abused drugs to be reported as designer drugs, recently. In Japan, the drug control laws prohibit not the only possession, transfer and illegal import of drugs, but also the usage of drugs described in the laws. For the above reason, examination of metabolites excreted in urine is widely carried out in forensic laboratories, since collection of blood by police officers and scientists has been banned, and this procedure is now only permitted to medical doctors. However, the structures of designer drugs are similar to one another. For this reason, the major metabolites of these kinds of drugs are the same between legal and illegal drugs. α-Methylfentanyl, one of the most widely abused fentanyl analogues, the itake of α-methylfentanyl could not be proved by major metabolites. In this study using rats as an experimental animal, four novel metabolites, such as ω and ω-1 hydroxy α-methylfentanyls (M4, 19 and M5, 20), p-aromatic hydroxy α-methylfentanyl (M6, 23), and p-aromatic hydroxy and ω hydroxy and α-methylfentanyl (M7, 24) were found in rat urine. To find these metabolites that reflect the original dosed drug, the usage of α-methylfentanyl are proved. From the detection of major metabolites, nor-fentanyl (M1, 6), ω-hydroxy nor-fentanyl (M2, 13) and ω-1 hydroxy nor-fentanyl (M3, 18) it is insufficient to prove the abuse of α-methylfentanyl. For the accurate identification to prove these designer drugs, the structures of abused drugs are similar to each other, in the examination of metabolites in urine, only detection of major metabolites is insufficient, minor metabolites that reflect the dosed drug structure is indispensable.

Furthermore, one of the reasons to explain the frequently reported fatal cases was due to not only over dose but also narrow range of ED_{50} and LD_{50}.[22)]

REFERENCES

1) Stinson, S. (1981) Structure of bogus "China White" solved. *Chem. Eng. News*, **19**, 71–72.
2) Kram, T. C., Cooper, D. A. and Allen, A. C. (1981) Behind the identification of China White. *Anal. Chem.*, **53**, 1379A–1386A.
3) Cheng, M. T., Kruppa, G. H. and McLafferty, F. W. (1982) Structural information from tandem mass spectrometry for China White and related fentanyl derivatives. *Anal. Chem.*, **54**, 2204–2207.
4) Baum, R. M. (1985) New variety of street drugs poses growing problem. Designer drugs—analogs of compounds with proven pharmacological activity made by underground chemists—present novel challenges to law enforcement officials, legislators, and scientists. *Chem. Eng. News*, **9**, 7–16.

5) Shafer, J. (1985) Designer drugs. *Science*, **85**, 60–67.
6) Cooper, D. A, Jacob, M. and Allen, A. C. (1986) Identification of fentanyl derivatives. *J. Forensic Sci.*, **31**, 511–528.
7) Suzuki, S., Inoue, T. and Kashima, C. (1986) Studies on 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) and related compounds. I. Spectroscopic and chromatographic analysis of 3-methylfentanyl and α-methylfentanyl. *Chem. Pharm. Bull*. (Tokyo), **34**, 1340–1343.
8) Suzuki, S. (1989) Studies on fentanyl and related compounds. II. Spectrometric discrimination of five mono methylated fentanyl isomers by gas chromatography/Fourier transform-infrared spectrometry. *Forensic Sci. Int*., **43**, 15–19.
9) Suzuki, S., Tsuchihashi, H. and Arimoto, H. (1989) Studies on 1-(2-phenethyl)-4-(N-propionylanilino)piperidine (fentanyl) and related compounds. III. Effect of methyl group introduction into fentanyl on sensitivity enhancement in gas chromatography with surface ionization detection. *J. Chromatogr. A*, **475**, 400–403.
10) Ohta, H., Suzuki, S. and Ogasawara, K. (1999) Studies on fentanyl and related compounds. IV. Chromatographic and spectrometric discrimination of fentanyl and its derivatives. *J. Anal. Toxicol.*, **23**, 280–285.
11) Van Wijngaarden, I. and Soudijn, W. (1968) The metabolism and excretion of the analgesic fentanyl (R 4263) by Wistar rats. *Life Sci*., **7**, 1239–1244.
12) Maruyama, Y. and Hosoya, E. (1969) Studies on the fate of fentanyl. *Keio J. Med.*, **18**, 59–70.
13) Goroumaru, T., Furuta, T., Baba, S., Yoshimura, N., Miyawaki, T., Sameshima, T. and Miyao, J. (1981) Metabolism of fentanyl in rats and man. *Anesthesiology*, **55**, A173.
14) National Police Agency (2006) *White Paper on Police* (*Annual report of police activities in Japan*), Government of Japan.
15) Van Rooy, H. H., Vermeulen, N. P. E. and Bovill, J. G. (1983) The assay of fentanyl and its metabolites in plasma of patients using gas chromatography with alkali flame ionization detection and gas chromatography-mass spectrometry. *J. Chromatogr. B*, **223**, 85–93.
16) Goroumaru, T., Matsuura, H., Furuta, T., Baba, S., Yoshimura, N., Miyawaki, T. and Sameshima, T. (1982) Identification of fentanyl metabolites in rat urine by gas chromatography-mass spectrometry with stable-isotope tracers. *Drug Metab. Dispos.*, **10**, 542–546.
17) Goroumaru, T., Matsuura, H., Yoshimura, N., Miyawaki, T., Sameshima, T., Miyao, J., Furuta, T. and Baba, S. (1984) Identification and quantitative determination of fentanyl metabolites in patients by gas chromatography-mass spectrometry. *Anesthesiology*, **61**, 73–77.
18) Goroumaru, T., Katashima, K., Matsuura, H. and Yoshimura, N. (1985) Metabolism of fentanyl in isolated hepatocytes from rat and guinea pig. *Chem. Pharm. Bull.* (Tokyo), **33**, 3922–3928.
19) Feierman, D. E. and Lasker, J. M. (1996) Metabolism of fentanyl, a synthetic opioid analgesic, by human liver microsomes. Role of CYP3A4. *Drug. Metab. Dispos.*, **24**, 932–939.
20) Labroo, R. B., Paine, M. F., Thummel, K. E. and Kharasch, E. D. (1997) Fentanyl metabolism by human hepatic and intestinal cytochrome P-450 3A4: implications for interindividual variability in disposition, efficacy and drug interactions. *Drug Metab. Dispos*., **25**, 1072–1080.
21) Schuttler, J. and White, P. F. (1984) Optimization of the radioimmunoassays for measuring fentanyl and alfentanil in human serum. *Anesthesiology*, **61**, 315–320.
22) Higashikawa, Y. and Suzuki, S. (2008) Studies on 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) and its related compounds. VI. Structure-analgesic activity relationship for fentanyl, methyl-substituted fentanyls and other analogues. *Forensic Toxicology*, **26**, 1–5.