Identification of *N*-Methyl-4-(3,4-Methylenedioxyphenyl) Butan-2-Amine, Distributed as MBDB

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***N*-methyl-1-(3,4-methylenedioxyphenyl)butan-2-amine (MDP-2-MB, MBDB) is a new homologue of *N*-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine (MDMA), which is strictly controlled as a narcotic. As part of our continuous survey on illegal designer drugs in the Japanese market, we found that *N*-methyl-4-(3,4-methylenedioxyphenyl)butan-2-amine (MDP-3-MB, HMDMA) was being sold as MBDB. As this is the first time that HMDMA has been revealed to be in market distribution, and its physico-chemical data is thus far unreported, we describe the structure elucidation of HMDMA and comparative analysis with related compounds.**

Key words —— *N*-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine homologue, structure elucidation, qualitative analysis

INTRODUCTION

Amphetamine, methamphetamine and related designer drugs 1-(3,4-methylenedioxyphenyl) propan-2-amine (MDA), *N*-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine (MDMA), *N*-ethyl-1-(3,4-methylenedioxyphenyl)propan-2-amine (MDEA), *N*-methyl-1-(3,4-methylenedioxyphenyl) butan-2-amine (MDP-2-MB, MBDB) and 2-methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one (methylone) are phenylalkylamine analogues (Fig. 1) which belong to a wider group of central nervous system stimulants. These six compounds, apart from methylone, are strictly controlled by the Narcotics and Psychotropic Control Law in Japan since April 2006, particularly MBDB.

As part of our continuous survey on illegal designer drugs in the Japanese market, we focused our attention on 3,4-methylenedioxyphenyl compounds. In the survey, we found a phenylbutanamine derivative which is distinct from MBDB, although the drug may be labeled as containing MBDB. After spectroscopic analysis, the structure was identified as *N*-methyl-4-(3,4-methylenedioxyphenyl)butan-2-amine (MDP-3-MB,[1] HMDMA). As far as we know, this is the first time that HMDMA has been shown to be in market distribution, although several reports have described its pharmacological activity and analytical methods.

Little is known about the hallucinogenic activity of HMDMA, but some qualitative differences in pharmacological activity have been observed. Davis and Borne[2] have compared the acute toxicities of MDA and MDMA with those of 4-(3,4-methylenedioxyphenyl)butan-2-amine (HMDA) and HMDMA. They found that HMDA and HMDMA were equal or greater in toxicity than MDA and MDMA in mice, suggesting that HMDA and HMDMA constitute no less of a hazard for acute toxicity in humans than MDA. Bronson *et al*.[3] examined the effects of *d*-amphetamine and the designer drugs MDMA, HMDMA, 1-(3,4-methylenedioxyphenyl)butan-2-amine and 2-(3,4-methylenedioxyphenyl)ethan-1-amine in chick embryos and young chickens. HMDMA had no effects on motility but produced effects such as tremor, flat body posture, loss of righting reflex and liver weight reduction. They also reported that the higher doses (30–60 mg/kg) of HMDMA elicited clonic seizures in rats.[4] Physico-chemical data for HMDMA has not yet been reported. An analytical procedure for HMDMA has been reported only by Noggle *et al*.[5] *N*-substituted 4-(3,4-methylenedioxyphenyl)butan-2-amines were separated via reversed-phase liquid chromatographic methods, partially based on comparative analysis with MDA, and the electron impact mass spectra of these compounds were determined using a capillary gas chromatography-mass spectrometry (GC-MS) system.

In this study, we deal with the structural identification of HMDMA by spectroscopic analysis. In addition, we describe comparative analyses of HMDMA and related substances amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB and methylone from the viewpoint of drug surveillance.

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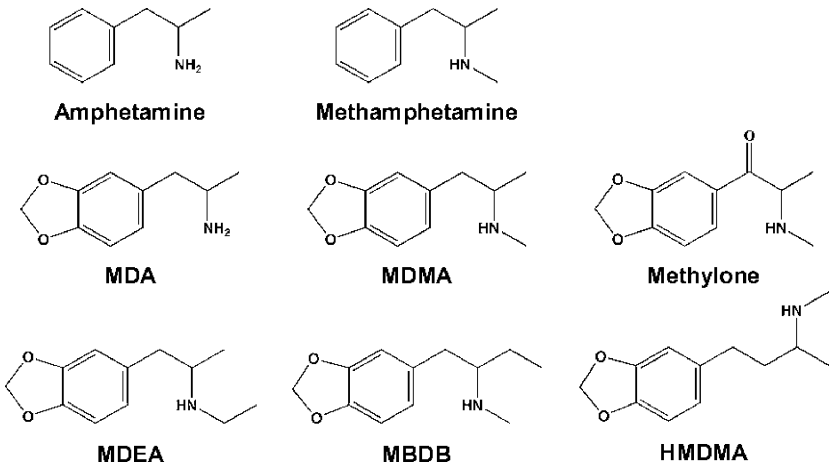

Fig. 1. Chemical Structures of Phenylamine Drugs

MATERIALS AND METHODS

HMDMA was purchased via the Internet in Japan. MBDB hydrochloride was purchased from Cerilliant™ (TX, U.S.A.). Standards of amphetamine, methamphetamine, MDA, MDMA, MDEA, and methylone were obtained from the collection of standards of the Division of Pharmacognosy, Phytochemistry and Narcotics at the National Institute of Health Sciences, Tokyo, Japan. The other chemicals used were of reagent grade and were purchased from Pure Chem. Ind. Ltd. (Osaka, Japan).

High resolution-time of flight mass spectrometry (HR-TOFMS) was performed on a Waters Micromass® LCT Premier™ mass spectrometer (Waters, MA, U.S.A.). Infra-red spectra were recorded on a JEOL JIR-SPX200 spectrophotometer (JEOL, Tokyo, Japan).

NMR —— ^{1}H and ^{13}C NMR data were acquired at room temperature, on a JEOL ECA 600 spectrometer (JEOL) operating at 600.17 and 150.91 MHz, respectively. Methanol (MeOH)-d_4 was used as a solvent. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane as an internal reference; coupling constants (J) are given in Hz. The proton and corresponding carbon signals were assigned using DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

GC-MS —— GC-MS analysis was carried out using a Hewlett-Packard® HP G1800A GCD system (CA, U.S.A.) consisting of a gas chromatograph and an electron ionization detector (EID). The instrument is controlled by a data system that consists of an HP Vectra 486 personal computer. Chromatographic separation was carried out on a 30 m × 0.25 mm i.d. fused silica capillary column HP-35MS (100% dimethylpolysiloxane; film thickness 0.25 mm; Ref. 13668). The injector was used in split mode (1 : 1) at 200°C, and the gas-chromatograph oven temperature was programmed as follows: the initial temperature was 60°C for 1 min, and the temperature was then raised at a rate of 20°C/min to 280°C; this temperature was maintained for 10 min. Helium was used as the carrier gas, at a flow rate of 1 ml/min. The detector (EID) was operated in positive electron impact mode with the ion source temperature set at 250°C, the ionization voltage at 70 eV and the mass spectrometer (quadrupole) used in scan mode. Spectra were recorded from m/z 40 to 500 at a rate of 1 scan/sec.

For GC analysis, amounts of 0.1 mg of each powder were placed into screw-capped tubes and dissolved in 1.0 ml of 1% ammonia (NH_3) aq. Analytes were extracted from NH_3 solution using 1.0 ml of ethyl acetate; the upper organic layers were then transferred into other screw-capped tubes for GC (FID and MS-EI) analysis of the native molecules. Each standard sample was also prepared by same procedure.

Liquid Chromatography-Mass Spectrometry (LC-MS) —— LC-MS analysis was performed on a Agilent 1100 series HPLC system (Agilent Technology, CA, U.S.A.) consisting of a Series 200 pump, a "hot pocket'' column oven, a Series 200 autosampler and a SCIEX API 2000 MS instrument (Applied Biosystems, Stockholm, Sweden) equipped with an electrospray interface (Turbo Ion Spray). Ion spray voltage was set to 3500 V. Nitrogen was used as a nebulizer gas (50 psi), auxiliary gas (50 psi heated to 300°C), curtain gas (30 psi) and

collisionally activated dissociation gas (set on 5). We used a 150 × 2.1 mm Atlantis dC_{18} analytical column with 5-μm particle size (Waters, MA, U.S.A.). The mobile phase consisted of a 10 mM ammonium formate buffer (pH 4.0) and acetonitrile. The system was run in a linear gradient from 85% (0 min) to 70% aqueous phase (35 min). The total flow rate was 0.3 ml/min. The column oven was set at 40°C. A 1-μl aliquot of the sample was injected. For the detection system, a tandem setting of a photo diode array detector (PDA) and a mass detector (MSD) was adopted. Mass analysis by ESI was conducted in positive mode. Chromatograms were evaluated using Analyst v1.1. Each sample was accurately adjusted to form a 0.1 mg/ml MeOH solution. A standard working solution was prepared by mixing an aliquot of each stock solution for simultaneous analyses of stimulants.

Thin Layer Chromatography (TLC) —— TLC was carried out using silica gel 60 F_{254} pre-coated plates (Merck, Darmstadt, Germany). The mobile phases for analytes were MeOH / 25% NH3 *aq*. solution (100/0.15) and chloroform ($CHCl_3$)/acetone/MeOH/25% NH_3 *aq*. (15/12/3/0.1). Ten μl of 0.1 mg/ml solution was spotted in a line across a silica gel sheet, approximately 30 mm from the bottom edge. The chromatograms were developed up to 100 mm at room temperature. After evaporation of the mobile phase in a flow of warm air, the spots on the chromatograms were observed under UV light (254 nm).

Color Tests —— Simon and Marquis reagents were purchased from Kanto Chemical Co. Ltd (Tokyo, Japan). Mandelin reagent was prepared by dissolving 0.5 g of ammonium vanadate 1.5 ml of water and diluting with 100 ml of concentrated sulfuric acid in a volumetric flask. The hydrochloric acid salt of each analyte (1.0 mg) was added to the wells on the porcelain test plate, and one or two drops of reagent(s) were added to the drug using a Pasteur pipette. The final color was observed after 5 min.

RESULTS AND DISCUSSION

Structure Elucidation and Origin of HMDMA

The white powder *N*-methyl-4-(3,4-methylenedioxyphenyl)butan-2-amine, known as HMDMA, $[\alpha]_D$ 0.001° (c. 0.10, MeOH), showed a HR-TOFMS quasimolecular ion peak at *m*/*z* 208.1337 (calc. 208.1338) $[M + H]^+$, corresponding to the molecular formula $C_{12}H_{17}NO_2$. An IR absorption at 3404 cm^{-1} was attributed to an amino group. The ^1H NMR spectrum of HMDMA exhibited 16 non-exchangeable protons, including a tertiary methyl group (δ 2.66), a singlet methylene group (δ 5.89) and 1,3,4-trisubstituted aromatic ring [δ 6.69 (1H, dd, J = 1.4, 7.9 Hz, H6′), δ 6.73 (1H, d, J = 7.9 Hz, H5′), δ 6.75 (1H, d, J = 1.4 Hz, H2′)].[6] The ^{13}C NMR spectrum of HMDMA showed two methyl, three methylene (including a dioxygenated carbon (δ 102.2), a methine and six olefinic carbons (δ 109.2–149.3).[6] The presence of a 3,4-methylenedioxyphenyl group was evident from the NMR spectra, which showed HMBC correlations between the singlet methylene and the benzene carbon signals (δ 147.6, 149.3). The gross structure was finally determined by extensive 2D NMR experiments (Fig. 2). HMDMA was shown to exist as a racemic and acidic salt by optical rotation measurement and organic solvent transfer extracted with alkaline aqueous solution, respectively.

Noggle *et al*.[5] reported that availability of 1-(3,4-methylenedioxyphenyl)-2-propanone, which is a starting reagent for MDMA in clandestine laboratories, is restricted. If the commercially available ketone 1-(3,4-methylenedioxyphenyl)-3-butanone is used as an alternative starting reagent in the hopes of synthesizing MDMA, the resulting product is HMDMA.

Analyses of Phenylalkylamines

Simon, Marquis and Mandelin reagents were used for color tests to discriminate between the individual samples. MDA, MDMA, MDEA, MBDB and HMDMA take on similar colors to those of the reagents used because they do not have distinctive functional groups. It was noted that methylone, which possesses a carbonyl group, showed a unique color (Table 1).

In the TLC experiment, under the conditions described in Materials and Methods section, eight samples showed the R_f values listed in Table 2. It was difficult to discriminate MDMA from HMDMA using the solvent system A because of the similarity of their R_f values, as was the case for amphetamine, MDA, MDEA and MBDB using the solvent system B. These results suggest that the standard material of HMDMA is needed to distinguish it from other designer drugs in primary surveillance with the color test and TLC.

Ethyl acetate solutions of free base samples were used for GC-MS measurement. Each sample was well resolved under the conditions described (Fig. 3)

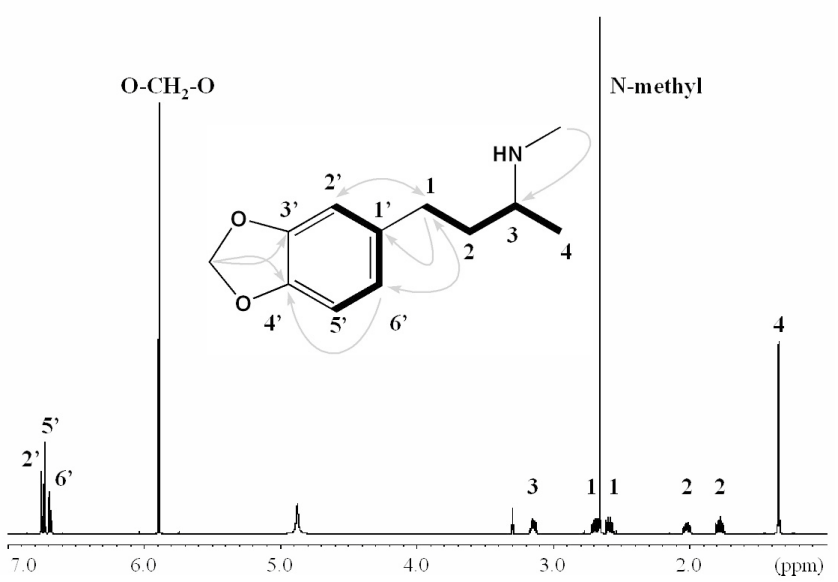

Fig. 2. ^{1}H NMR Spectrum of HMDMA Hydrochloride in MeOH-d_4

Assignments correspond to positions in the HMDMA molecule. Bold lines and arrows show ^{1}H-^{1}H COSY correlations and selected HMBC correlations, respectively.

Table 1. Results of Color Tests

Stimulant	Simon	Marquis	Mandelin
Amphetamine	not tested	orange/reddish brown	not tested
Methamphetamine	not tested	orange to brown	not tested
MDA	not tested	black	blackish purple
MDMA	not tested	black	blackish purple
MDEA	indigo (deep bluish purple)	blackish purple	blackish purple
MBDB	indigo (deep bluish purple)	blackish purple	blackish blue
HMDMA	indigo (deep blue)	blackish purple	blackish purple
Methylone	magenta	bright yellow	ocher

Table 2. R_f Values of Stimulants in Silica Gel TLC

Stimulant	System A	System B
Amphetamine	0.65	0.42
Methamphetamine	0.27	0.35
MDA	0.59	0.40
MDMA	0.16	0.29
MDEA	0.40	0.46
MBDB	0.33	0.43
HMDMA	0.23	0.28
Methylone	0.52	0.62

System A: $CHCl_3$: acetone : MeOH : 25% NH_3 *aq.* = 15 : 12 : 3 : 0.1. System B: MeOH : 25% NH_3 *aq.* = 10 : 0.15.

with specific fragments, ethylamine (*m*/*z* 44), *N*-methylethylamine (*m*/*z* 58), diethylamine or *N*-methylpropylamine (*m*/*z* 72), and 3,4-methylenedioxybenzyl (*m*/*z* 135) groups (Table 3), although the corresponding molecular ions were not observed.

The results of HPLC separation of the analytes are shown in Fig. 4. Using the analytical conditions described in MATERIALS AND METHODS, good separation of all of the drugs was confirmed in 15 min. The protonated molecular ions ($[M + H]^+$) were observed as base peak ions for all compounds investigated in this study (Table 4).

Since LC-MS and GC-MS provide information on molecular weight and partial structure, respectively, analysis using a combination of these methods should facilitate elucidation of the structure without the standard material. Therefore, a combination analysis method is recommended for surveillance of designer drugs.

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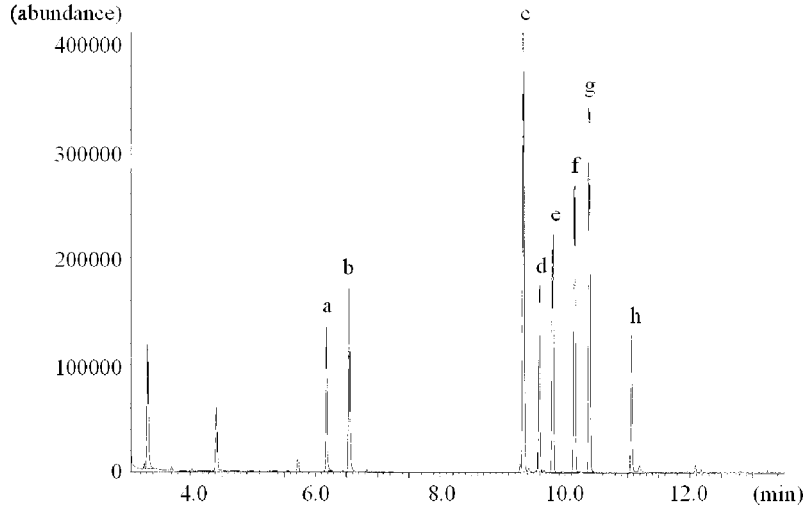

Fig. 3. Total Ion Chromatogram Obtained by GC-MS Analysis
Peak designations are as listed in Table 3.

Table 3. GC-MS Data of Stimulants

Peak	R.T. (min)	Stimulant	Five-peak index of mass spectra (% intensity)				
a	6.18	Amphetamine	44 (100)	91 (17.7)	65 (11.9)	42 (6.7)	51 (5.9)
b	6.55	Methamphetamine	58 (100)	91 (14.6)	65 (8.2)	56 (8.0)	42 (5.4)
c	9.35	MDA	44 (100)	136 (24.6)	77 (12.3)	135 (12.3)	51 (12.2)
d	9.60	MDMA	58 (100)	77 (8.2)	135 (7.9)	51 (7.0)	56 (4.8)
e	9.81	MDEA	72 (100)	44 (22.5)	135 (10.3)	77 (9.4)	51 (6.8)
f	10.16	MBDB	72 (100)	77 (7.8)	135 (7.6)	57 (7.5)	51 (6.7)
g	10.39	HMDMA	58 (100)	135 (22.8)	77 (10.4)	51 (7.5)	207 (6.7)
h	11.07	Methylone	58 (100)	63 (8.5)	65 (8.3)	56 (6.5)	149 (5.6)

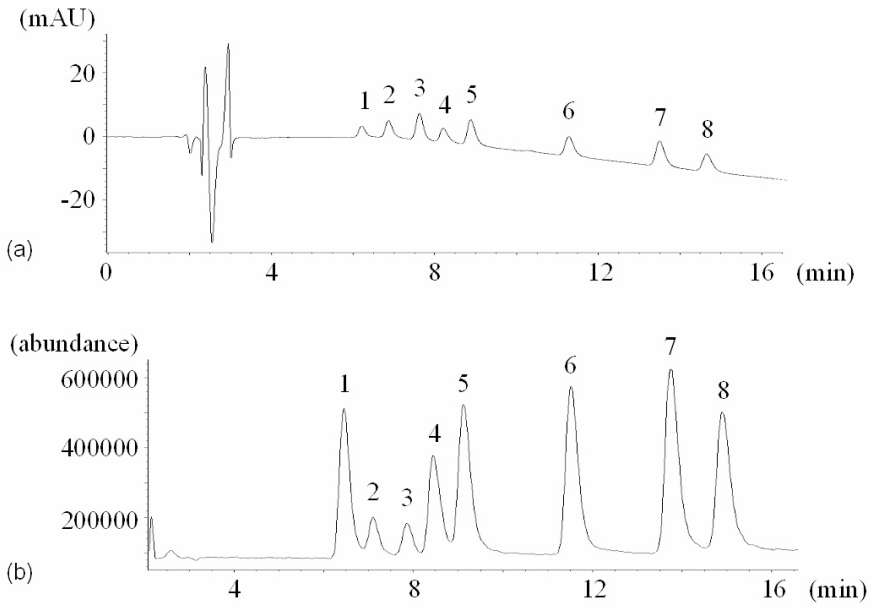

Fig. 4. HPLC Separation of Analytes
(a) UV (210 nm) detection, (b) MS (ESI) detection. Peak numbers are as listed in Table 4.

Table 4. LC-MS Data of Stimulants

Peak	R.T. (min, DAD)	Stimulant	$[M+H]^+$
1	6.2	Methylone	208
2	6.9	Amphetamine	136
3	7.6	MDA	180
4	8.2	Methamphetamine	150
5	8.9	MDMA	194
6	11.3	MDEA	208
7	13.5	MBDB	208
8	14.6	HMDMA	208

Health, Labor and Welfare, Japan.

REFERENCES

1) Baudot, P. H., Vicherat, A., Viriot, M. L. and Carré, M. C. (1999) Identification of *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), an homologue derivative of "ecstasy". *Analusis*, **27**, 523–532.
2) Davis, W. M. and Borne, R. F. (1984) Pharmacologic investigation of compounds related to 3,4-methylenedioxyamphetamine (MDA). *Subst. Alcohol Actions Misuse*., **5**, 105–110.
3) Bronson, M. E., Jiang, W., Clark, C. R. and DeRuiter, J. (1994) Effects of designer drugs on the chicken embryo and 1-day-old chicken. *Brain Res. Bull*., **34**, 143–150.
4) Bronson, M. E., Barrios-Zambrano, L., Jiang W., Clark, C. R., DeRuiter, J. and Newland, M. C. (1994) Behavioral and developmental effects of two 3,4-methylenedioxymethamphetamine (MDMA) derivatives. *Drug Alcohol Depend*., **36**, 161–166.
5) Noggle, F. T., Clark, C. R. and DeRuiter, J. (1989) Liquid chromatographic and mass spectral analysis of 1-(3,4-methylenedioxyphenyl)-3-butanqmines. *J. Chromatogr. Sci*., **27**, 240–243.
6) UV spectrum was recorded on a Shimadzu UV2100PC spectrophotometer (Kyoto, Japan). UV λ_{max}^{MeOH} (log ε): 202 (4.48), 235 (3.57) and 286 (3.62). ^{1}H and ^{13}C NMR assignments are as follows. ^{1}H NMR δ: 1.37 (3H, d, J = 6.5), 1.78 (1H, m), 2.02 (1H, m), 2.58 (1H, m), 2.66 (3H, s), 2.69 (1H, m), 3.15 (1H, m), 5.89 (2H, s), 6.69 (1H, dd, J = 1.4, 7.9), 6.73 (1H, d, J = 7.9), 6.75 (1H, d, J = 1.4); ^{13}C NMR δ: 15.9 (q), 30.6 (q), 32.0 (t), 36.0 (t), 56.1 (d), 102.2 (t), 109.2 (q), 109.7 (q), 122.3 (q), 135.4 (s), 147.6 (s), 149.3 (s).