

Simultaneous analysis of six novel hallucinogenic (tetrahydrobenzodifuranyl)aminoalkanes (FLYs) and (benzodifuranyl)aminoalkanes (DragonFLYs) by GC-MS, LC-MS, and LC-MS-MS

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Abstract Six novel hallucinogens classed as (tetrahydrobenzodifuranyl)aminoalkanes or (benzodifuranyl)aminoalkanes, which are known by the common names of “FLY” and “DragonFLY,” respectively, were synthesized. These compounds were simultaneously analyzed by gas chromatography (GC)-mass spectrometry (MS), liquid chromatography (LC)-MS, and LC-MS-MS. GC-MS analysis of their free bases was not satisfactory for both mass spectral and chromatographic measurements, and thus trifluoroacetyl (TFA) derivatization was employed. However, it was found that the usual TFA derivatization procedure using trifluoroacetic anhydride caused dehydrogenation of FLYs to the corresponding DragonFLYs. Therefore, TFA derivatization of FLYs was reinvestigated; the presence of triethylamine could almost inhibit such dehydrogenation. LC separation of the analytes was successfully achieved by using a phenyl-type semimicro column with methanol gradient elution, while 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-methylaminopropane (*N*-methyl-DOB-FLY) and 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane (DOB-FLY) were not separated on an octadecylsilica (ODS)-type column. Specific product ion spectra for all compounds were also obtained using LC-MS-MS, which enabled sensitive and reliable identification.

Keywords (Tetrahydrobenzodifuranyl)aminoalkanes · (Benzodifuranyl)aminoalkanes · FLY · DragonFLY · Hallucinogen · MS analysis

Introduction

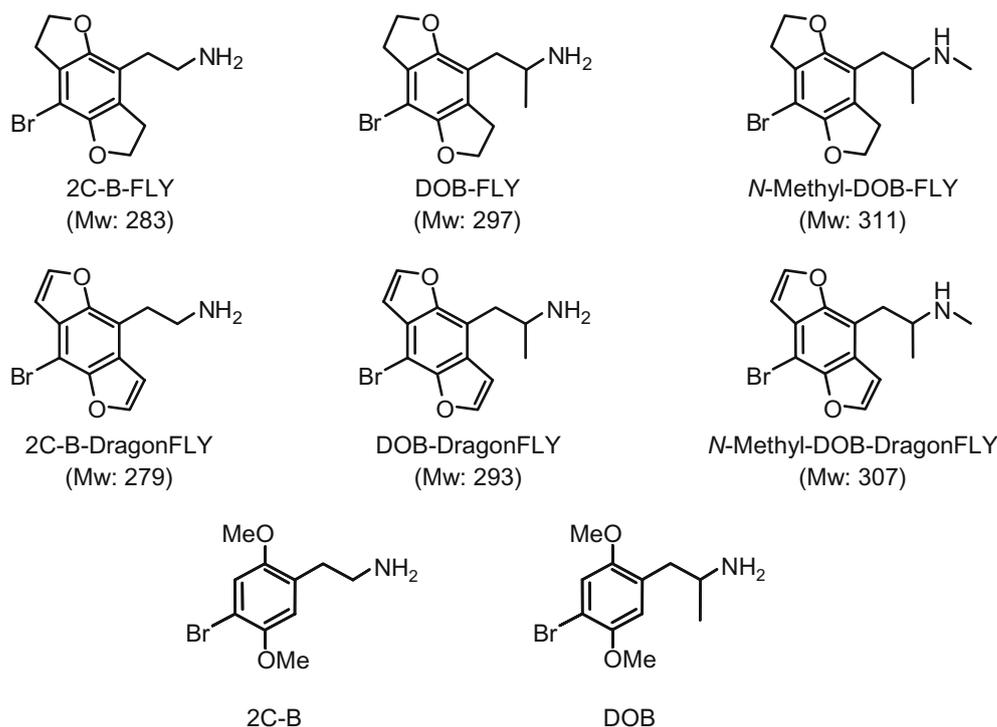
(Tetrahydrobenzodifuranyl)aminoalkanes and (benzodifuranyl)aminoalkanes, which are known by the street names of “FLY” and “DragonFLY,” are potent hallucinogens due to their characteristic dihydrofuran or difuran rings, respectively [1–3]. The typical name of FLY allegedly derives from the analogies of shapes between the insect fly and the chemical structures of (benzodifuranyl)aminoalkanes: two fly wing-like furan moieties attached to the central benzene ring, the fly head-like bromo-substituent, and the fly tail-like alkyl amino group [4]. Their structures are related to those of hallucinogens 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and 4-bromo-2,5-dimethoxyamphetamine (DOB) (Fig. 1).

Among these compounds, 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminoethane (2C-B-FLY) and 1-(8-bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane (DOB-DragonFLY) especially show extremely potent activities toward the 5-HT_{2A} receptor and strong hallucinogenic effects [2]. These compounds have recently been prepared in clandestine laboratories to be sold on underground drug markets, mainly in Europe. They are sometimes found in “blotter” being composed of small bits of paper, and are being sold as novel designer drugs on the Internet even in Japan [4–6].

These novel hallucinogens are slightly less potent than LSD [2], and their usual single dose is in the range

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Fig. 1 Chemical structures of FLYs [(tetrahydrobenzodifuranyl)-aminoalkanes], DragonFLYs [(benzodifuranyl)-aminoalkanes] and their analogues investigated in this study. 2C-B, 4-bromo-2,5-dimethoxyphenethylamine; DOB, 4-bromo-2,5-dimethoxyamphetamine



of 500–1000 µg, which causes extremely long active duration [7]. Thus, there are high risks of acute intoxication due to their overdose. In fact, human fatal poisoning cases involving DOB-DragonFLY have been reported in Scandinavia [8]. Moreover, potential risks of their abuse have been expanded worldwide, and reliable analytical methods for their identification should be established for drug enforcement to prevent their widespread abuse. However, there have been few reports on their analyses [4–6].

In this study, six compounds of authentic standards of 2C-B-FLY, DOB-DragonFLY, and their analogues were synthesized, and their mass spectral and chromatographic properties were demonstrated by gas chromatography (GC)-mass spectrometry (MS), liquid chromatography (LC)-MS, and LC-MS-MS.

Materials and methods

Chemicals and their preparation

2C-B-FLY, 1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane (DOB-FLY), 1-(8-bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminoethane (2C-B-DragonFLY), and DOB-DragonFLY were synthesized according to the previously proposed procedures [1–3,9]. 1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-methylaminopropane (*N*-methyl-DOB-FLY) and 1-(8-bromobenzo[1,2-b;4,5-

b']difuran-4-yl)-2-methylaminopropane (*N*-methyl-DOB-DragonFLY) were synthesized with slight modification of the method of Pizarro et al. [10]. The chemical structures of the six synthesized standards are shown in Fig. 1.

All synthesized standards were purified as their hydrochlorides, and their composition formulas were confirmed by high-resolution MS (MicroTOFII, Bruker Daltonics, Billerica, MA, USA) with an external calibration method. Chemical structures were confirmed by MS, infrared spectroscopy (Nicolet Magna FT-IR 3200; Thermo Fisher Scientific, Waltham, MA, USA), and proton nuclear magnetic resonance spectroscopy (Varian, Palo Alto, CA, USA). Every synthesized compound was ensured to be greater than 98% purity based on LC-MS analysis by the flow-injection method. Standard stock solutions of these compounds were prepared in distilled water at 10 mg/ml each, and stored at –20°C until analysis; they were diluted to appropriate concentrations immediately before use. Distilled water and LC-MS-grade methanol were used throughout the experiments. All other chemicals and reagents were of analytical grade or better purity purchased from Wako (Osaka, Japan).

Sample preparation for GC-MS

For analysis of the target compounds in the free forms by GC-MS, six kinds of stock standard solution were mixed, and extracted into ethyl acetate (EtOAc); 1 ml of

the mixture solution (hydrochloride salts) was adjusted to pH 9 with 28% aqueous ammonia, and then extracted with 0.5 ml of EtOAc two times. After centrifuging at 1500 g, the organic layer was isolated, and the combined extracts were dried with anhydrous Na₂SO₄. To avoid loss of the analytes, 10 µl of acetic acid was added to the extract. After evaporation to dryness under a gentle nitrogen stream at 40°C, the residue was reconstituted in 100 µl EtOAc. A 1-µl aliquot was automatically injected into the GC-MS system.

GC-MS analysis of the target compounds after trifluoroacetyl (TFA) derivatization was also tested; to avoid loss of the analytes, 10 µl of acetic acid was added to the 1-ml EtOAc extract described above. After evaporation to dryness under a gentle nitrogen stream at 40°C, the residue was subjected to TFA derivatization. After adding 50 µl of EtOAc and 200 µl of 1% (v/v) triethylamine (TEA) EtOAc solution to the residue, 50 µl of trifluoroacetic anhydride (TFAA) was added to the mixture. After reacting at room temperature for 10 min, 700 µl of EtOAc was added to the reaction mixture, which was then washed with 1 ml of distilled water. After centrifuging at 1500 g, the organic layer was isolated, and dried with anhydrous Na₂SO₄. The 500-µl organic phase was separated, and was carefully evaporated to dryness under a gentle nitrogen stream at 40°C, followed by reconstituting in 50 µl of EtOAc [1,3,9]. A 1-µl aliquot of each sample was automatically injected into the GC-MS system.

Sample preparation for LC-MS and LC-MS-MS

The mixture solution (hydrochlorides) was filtered through a 0.22-µm membrane filter, and a 10-µl aliquot was automatically injected into the LC-MS and LC-MS-MS systems.

GC-MS conditions

GC-MS was performed on a Shimadzu GCMS QP2010 Plus instrument (Shimadzu, Kyoto, Japan) with a fused-silica capillary column DB-1MS, DB-5MS, or DB-17MS (30 m × 0.25 mm i.d.; film thickness, 0.25 µm; J&W Scientific, Folsom, CA, USA). The temperature program consisted of the initial temperature of 80°C held for 1 min, followed by a linear ramp up to 320°C at 15°C/min. The inlet temperature was set at 250°C, and the injection was set in the splitless mode. The carrier gas was high-purity helium at a flow rate of 1.0 ml/min. The MS detector parameters used were: interface temperature, 250°C; ion-source temperature, 200°C; ionization mode, electron ionization (EI); ionization voltage, 70 eV; scan time, 0.5 s/scan; scan range, *m/z* 29–550.

LC-MS conditions

LC-MS was carried out on a Shimadzu LCMS-QP2010 EV quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. An L-column ODS2 semimicro column (particle diameter, 5 µm; 150 mm × 1.5 mm i.d.; Chemicals Evaluation and Research Institute, Tokyo, Japan) and a CAPCELL PAK Phenyl semimicro column (particle diameter, 5 µm; 150 mm × 1.5 mm i.d.; Shiseido, Tokyo, Japan) were used for chromatographic separation. The analytes were separated by linear-gradient elution with 10 mM ammonium formate buffer (adjusted to pH 3.5 with formic acid) and methanol (25%–50%, v/v) at a flow rate of 0.15 ml/min under a column temperature at 40°C, and the entire flow of the eluate was introduced into the ESI interface. ESI-MS was performed in the positive mode. Other operating parameters were: nebulizer nitrogen gas flow rate, 1.5 l/min; curved desolvation line (CDL) voltage, 25 V; Q-array bias; 5 V; CDL temperature, 250°C; ion-source temperature, 200°C; detection mode, selected ion monitoring (SIM) at *m/z* 284 for 2C-B-FLY, *m/z* 298 for DOB-FLY, *m/z* 312 for *N*-methyl-DOB-FLY, *m/z* 280 for 2C-B-DragonFLY, *m/z* 294 for DOB-DragonFLY, and *m/z* 308 for *N*-methyl-DOB-DragonFLY.

LC-MS-MS conditions

LC-MS-MS was operated on an API 3200 QTRAP system (Applied Biosystems/MDS Analytical Technologies, Concord, ON, Canada). The QTRAP analyzer combines a fully functional triple-quadrupole with an ion-trap mass spectrometer within the same platform. LC separation of the analytes was performed using a Prominence Series LC pump (Shimadzu). A CAPCELL PAK phenyl semimicro column was also used for chromatographic separation, and the analytes were chromatographed under exactly the same condition as described in the above LC-MS conditions. Nitrogen was used as nebulizer gas and collision gas. The MS parameters [declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), and collision cell exit potential (CXP)] were optimized by flow-injection analysis for each compound. The optimized parameters are shown in Table 1. Other parameters of the tandem MS detector were: scan mode, enhanced product ion scan (EPI) mode and selected reaction monitoring (SRM) mode; scan range for EPI, *m/z* 50–350; ion spray voltage, 5500 V; curtain gas, 10.0 (arbitrary units); GS1 and GS2, 345 and 552 kPa, respectively; probe temperature, 500°C; collision energies (CEs) for EPI, 15 and 40 eV; CEs for SRM, 19 eV for 2CB-FLY, 17 eV for DOB-FLY, 21 eV

Table 1 Precursor ions and mass spectral detector parameters

| Compound | Precursor ion (m/z) | DP (V) | EP (V) | CEP (V) | CXP (V) |
|----------------------------|-------------------------|--------|--------|---------|---------|
| 2C-B-FLY | 284 | 26 | 7.0 | 16 | 4.0 |
| DOB-FLY | 298 | 21 | 4.0 | 12 | 4.0 |
| <i>N</i> -Me-DOB-FLY | 312 | 41 | 6.5 | 12 | 4.0 |
| 2C-B-DragonFLY | 280 | 26 | 4.5 | 12 | 4.0 |
| DOB-DragonFLY | 294 | 26 | 5.0 | 10 | 4.0 |
| <i>N</i> -Me-DOB-DragonFLY | 308 | 31 | 7.5 | 16 | 4.0 |

DP, Declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CXP, collision cell exit potential; 2C-B, 4-bromo-2,5-dimethoxyphenethylamine; FLY, (tetrahydrobenzodifuranyl)aminoalkane; DOB, 4-bromo-2,5-dimethoxyamphetamine; DragonFLY, (benzodifuranyl)aminoalkanes

Table 2 Retention indices of FLY and DragonFLY compounds on different column types

| Parent compound | DB-1MS | | DB-5MS | | DB-17MS | |
|----------------------------|-----------|----------------|-----------|----------------|-----------|----------------|
| | Free base | TFA derivative | Free base | TFA derivative | Free base | TFA derivative |
| 2C-B-FLY | 2133 | 2257 | 2226 | 2309 | 2840 | 2860 |
| DOB-FLY | 2168 | 2243 | 2226 | 2291 | 2798 | 2780 |
| <i>N</i> -Me-DOB-FLY | 2204 | 2336 | 2259 | 2380 | 2804 | 2900 |
| 2C-B-DragonFLY | 2029 | 2137 | 2081 | 2189 | 2586 | 2660 |
| DOB-DragonFLY | 2038 | 2127 | 2087 | 2156 | 2560 | 2580 |
| <i>N</i> -Me-DOB-DragonFLY | 2081 | 2230 | 2124 | 2274 | 2574 | 2720 |

for *N*-methyl-DOB-FLY, 17 eV for 2C-B-DragonFLY, 15 eV for DOB-DragonFLY, and 19 eV for *N*-methyl-DOB-DragonFLY. The ions at m/z 284, 298, 312, 280, 294, and 308 corresponding to the protonated molecules of 2C-B-FLY, DOB-FLY, *N*-methyl-DOB-FLY, 2C-B-DragonFLY, DOB-DragonFLY, and *N*-methyl-DOB-DragonFLY, respectively, were selected as precursor ions. SRM transitions used were: m/z 284→267 for 2C-B-FLY, m/z 298→281 for DOB-FLY, m/z 312→281 for *N*-methyl-DOB-FLY, m/z 280→263 for 2C-B-DragonFLY, m/z 294→277 for DOB-DragonFLY, and m/z 308→277 for *N*-methyl-DOB-DragonFLY.

Results and discussion

GC-MS analyses

To investigate GC separation of the six compounds of FLY and DragonFLY, three different column phases (DB-1MS, DB-5MS, and DB-17MS) were tested under the same operating conditions as described in “Materials and methods” [11,12]. The elution order of the six compounds was different among the columns used, and their calculated Kovats indices [13] are listed in Table 2.

As an example, the total ion chromatogram (TIC) and mass chromatograms obtained from the underivatized compounds by using a DB-17MS column are shown in Fig. 2a. All analytes appeared as tailing peaks with all

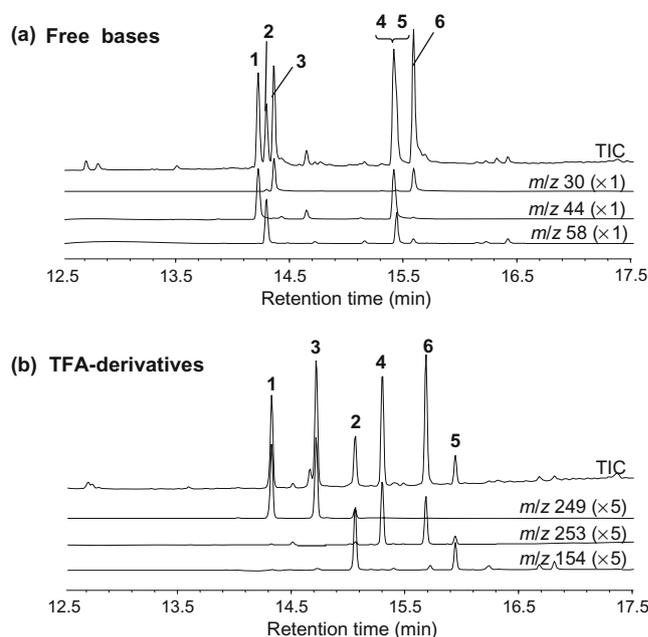
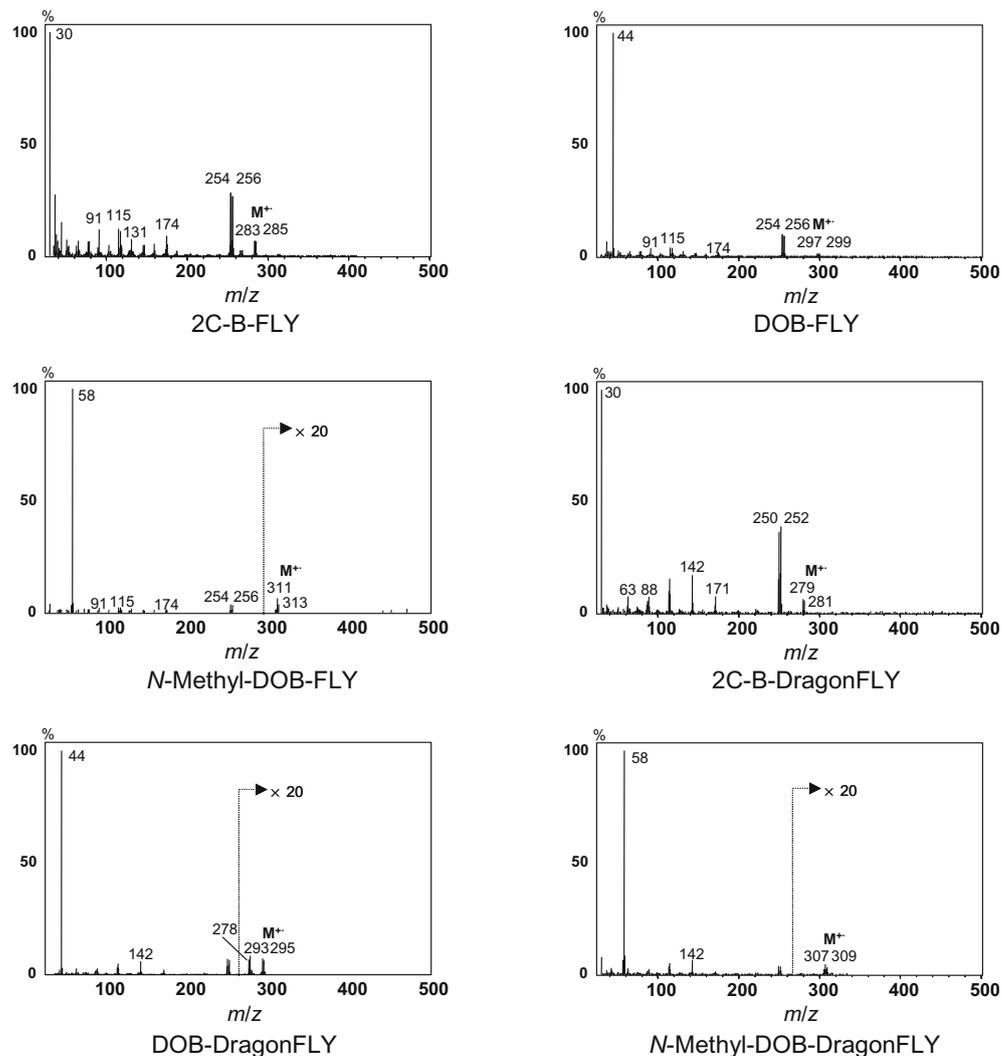


Fig. 2a, b Total ion chromatogram (TIC) and mass chromatograms obtained from the mixture of the standards (10 μ g/ml each) by gas chromatography-mass spectrometry (GC-MS) using a DB-17MS capillary column. **a** Free bases, **b** trifluoroacetyl (TFA) derivatives. Peaks: 1, DOB-DragonFLY; 2, *N*-Me-DOB-DragonFLY; 3, 2C-B-DragonFLY; 4, DOB-FLY; 5, *N*-Me-DOB-FLY; 6, 2C-B-FLY

columns used, indicating their relatively strong interaction with the stationary phases. The noticeable adsorption of 2C-B-FLY and 2C-B-DragonFLY to GC glass inlet was also observed, which made their limits of detec-

Fig. 3 Electron ionization mass spectra of the free bases of the six analytes



tion (LODs) higher (0.5 and 0.3 $\mu\text{g}/\text{ml}$, respectively, in the scan mode). The same phenomenon was reported for phenethylamine-type compounds like 2C-T-7 [14]. The LODs of other compounds were 0.1 $\mu\text{g}/\text{ml}$ each in the scan mode.

The EI-mass spectra of free-base forms of the analytes showed somewhat poor fragmentation profiles for their identification. As shown in Fig. 3, each EI mass spectrum of the analytes was characterized by the intense iminium ions at m/z 30, 44, and 58 produced by an α -cleavage reaction of amines, and the relatively small fragment ions at m/z 254 and 256 for FLY compounds generated by α -cleavage of benzyl bonds [15,16].

Our previous studies on various phenethylamines showed that TFA derivatization was effective not only for their mass spectral identification but also for improvement of their chromatographic properties [11,12,17–19]. TFA derivatization was also expected to suppress adsorption of 2C-B-FLY to the GC glass inlet, because

such adsorption of phenethylamines would be due to their highly polar amino moieties [14,20]. Their TFA derivatization was then examined according to the common method with TFAA [21]. However, FLY compounds were found to be unexpectedly dehydrogenated to their corresponding DragonFLY compounds during the derivatization (Fig. 4). This phenomenon was observed even when the derivatization was carried out at room temperature (Fig. 4b). In contrast, such dehydrogenation was not observed in the process of TFA derivatization in the synthesis of the authentic standards of DragonFLYs according to the previously proposed procedure [3,9]. It suggested that this dehydrogenation was due to the excess amounts of trifluoroacetic acid derived from TFAA, which might convert FLY-compounds into quinone-like forms acting as oxidants [22]. Thus, TEA was added as a trap reagent for a trifluoroacetic acid, and TFA derivatization was reexamined. In the preliminary experiments, an exothermic

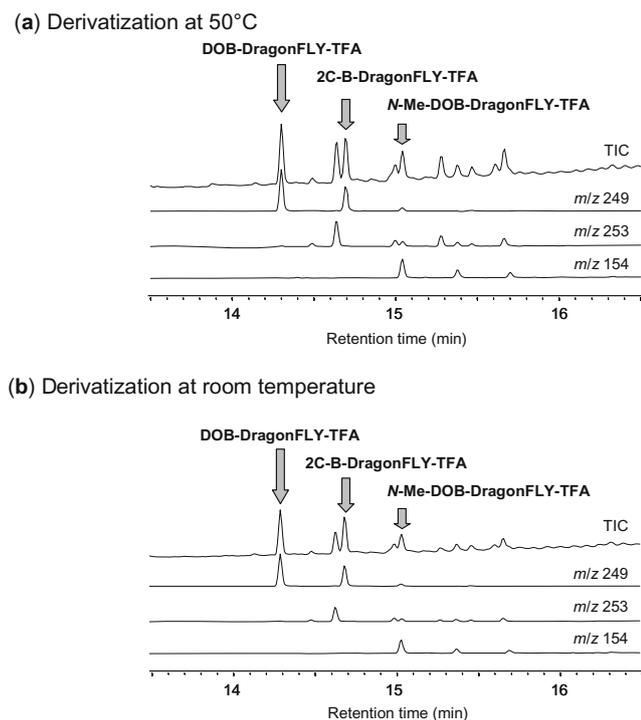


Fig. 4a, b Total ion chromatogram (TIC) and mass chromatograms by GC-MS obtained from FLY compounds after TFA derivatization according to the common method without addition of triethylamine

reaction occurred when mixing equimolar amounts of TEA and TFAA, which resulted in a nonvolatile yellowish residue; the product was detected as an extremely large broad peak in chromatograms by GC-MS. To minimize the generation of the residue, both amounts and concentrations of TEA to be added were further examined. We found that the generation of the nonvolatile residue was formed mainly depending not on absolute amounts of TEA but on the concentrations of TEA in the reaction solution. On the basis of the results, we finally optimized the derivatization procedure as described in “Materials and methods,” where most of the FLY-compounds were not dehydrogenated. It was, however, difficult to prevent their dehydrogenation completely.

All TFA derivatives of the analytes were sufficiently separated on each of the columns used (Fig. 2b). In addition, their peak shapes became extremely sharp, and their LODs were improved in comparison with those of their free bases; the LODs of TFA derivatives of the analytes in the scan mode were 20 ng/ml for 2C-B-FLY and 2C-B-DragonFLY, and 10 ng/ml for other compounds. The calculated Kovats indices [13] are also listed in Table 1.

As shown in Fig. 5, every EI mass spectrum of TFA derivatives showed typical and good fragmentation pat-

terns in comparison with those of their free bases (Fig. 3). The mass spectra of the TFA derivatives of 2C-B-FLY, DOB-FLY, 2C-B-DragonFLY, and DOB-DragonFLY were characterized by the intense ions at m/z 253 and 255 (for FLYs) and at m/z 249 and 251 (for DragonFLYs) generated by benzyl cleavage. The molecular ions and their isotopic ions due to Br were observed at m/z 379 and 381 for 2C-B-FLY-TFA, m/z 393 and 395 for DOB-FLY-TFA, m/z 375 and 377 for 2C-B-DragonFLY-TFA, and m/z 389 and 391 for DOB-DragonFLY-TFA, respectively. On the other hand, the mass spectra of the TFA derivatives of the *N*-methyl-compounds such as *N*-methyl-DOB-FLY and *N*-methyl-DOB-DragonFLY were characterized by the intense ions at m/z 154 produced by an α -cleavage reaction of the amines, and the typical fragment ions at m/z 110 for *N*-methylamines [12,15].

LC-MS analyses

On the basis of our previous LC-MS procedures for various phenethylamine-type designer drugs [14,15,23–25], an ODS-type semimicro column was first chosen for separation. As shown in Fig. 6a, DOB-FLY and *N*-methyl-DOB-FLY could not be separated with the ODS column. Thus, we tested a phenyl-type semimicro column. Figure 6b shows mass chromatograms obtained with the phenyl-type column for the six compounds. All analytes were well separated under the linear gradient conditions described in “Materials and methods.” Such unique chromatographic properties of the phenyl-type column would be due to the strong π - π electron interaction between the phenyl groups of the stationary phase and the analytes.

In the ESI mass spectra of the analytes, the protonated molecular ions were observed as base peaks for all compounds. The LODs of the analytes in the SIM mode were defined by the lowest concentration detectable with a signal-to-noise ratio of 3, and these were estimated to be 5 ng/ml for *N*-methyl-DOB-FLY and *N*-methyl-DOB-DragonFLY, and 10 ng/ml for other compounds. Thus, LC-MS may be advantageous over GC-MS, because no tedious sample pretreatment or derivatization was required for LC-MS, and no dehydrogenation of FLY-compounds was observed.

LC-MS-MS analyses

As described above, LC-MS was advantageous for analysis of FLY and DragonFLY compounds in some respects. However, mass spectral identification is, of course, most important, and it is most preferable to obtain good product ion spectra, which are more iden-

Fig. 5 Electron ionization mass spectra of the TFA derivatives of the six analytes

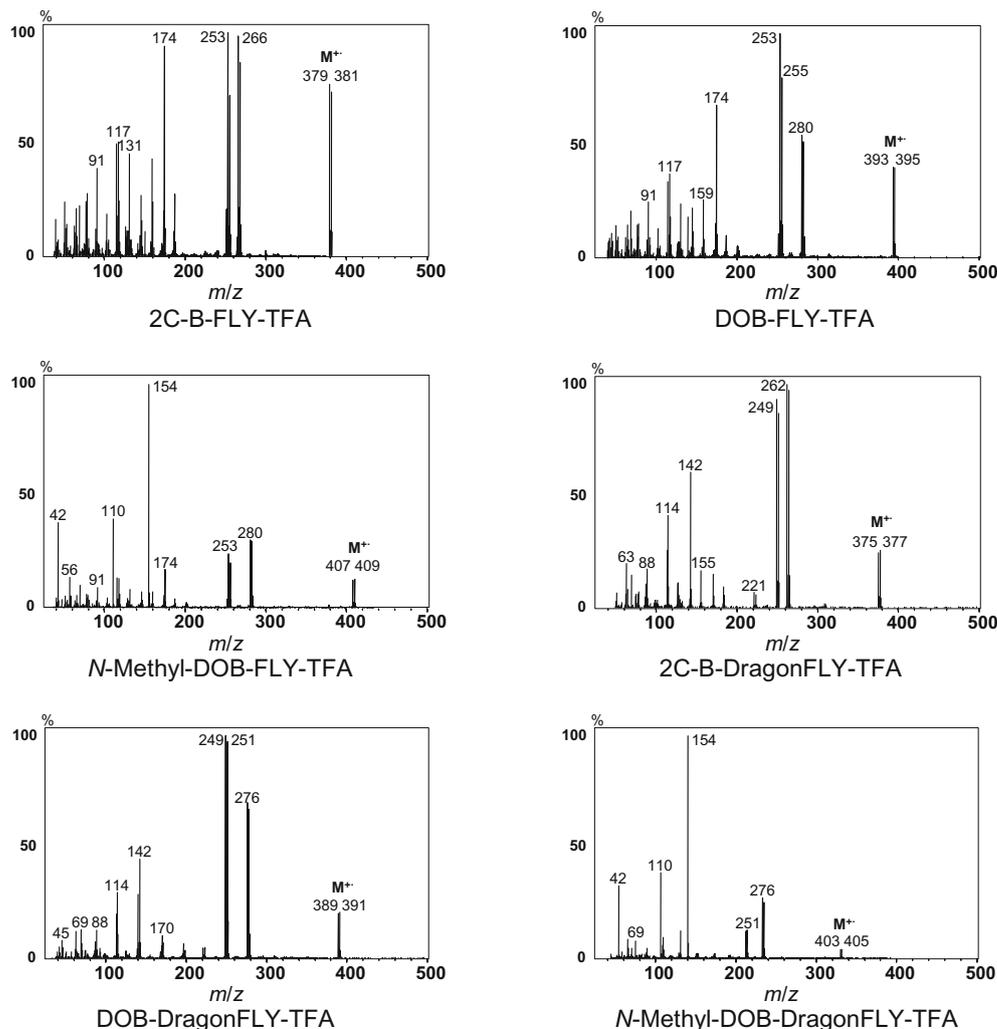


Fig. 6a, b Mass chromatograms obtained from the mixture of the standards (0.5 µg/ml each) by liquid chromatography-mass spectrometry (LC-MS). Separation with **a** an ODS-type column, and **b** a phenyl-type column. Peaks: 1, 2C-B-FLY; 2, DOB-FLY; 3, N-methyl-DOB-FLY; 4, 2C-B-DragonFLY; 5, DOB-DragonFLY; 6, N-methyl-DOB-DragonFLY

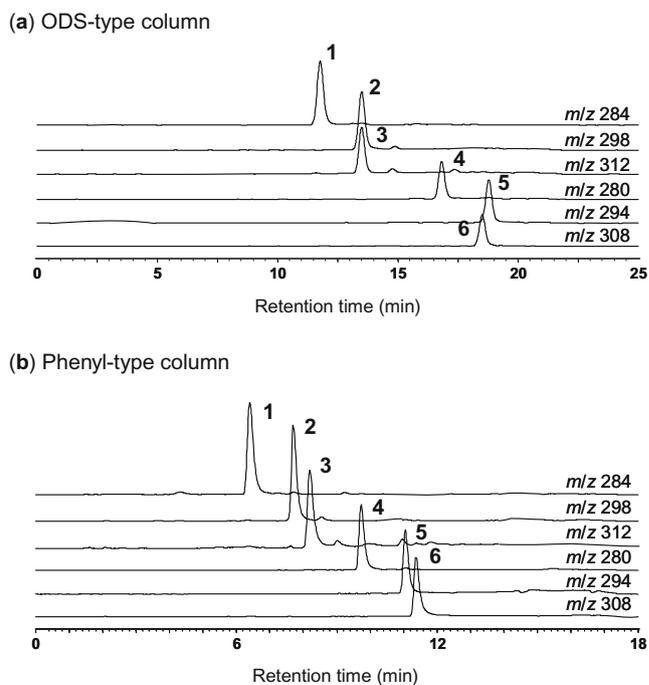
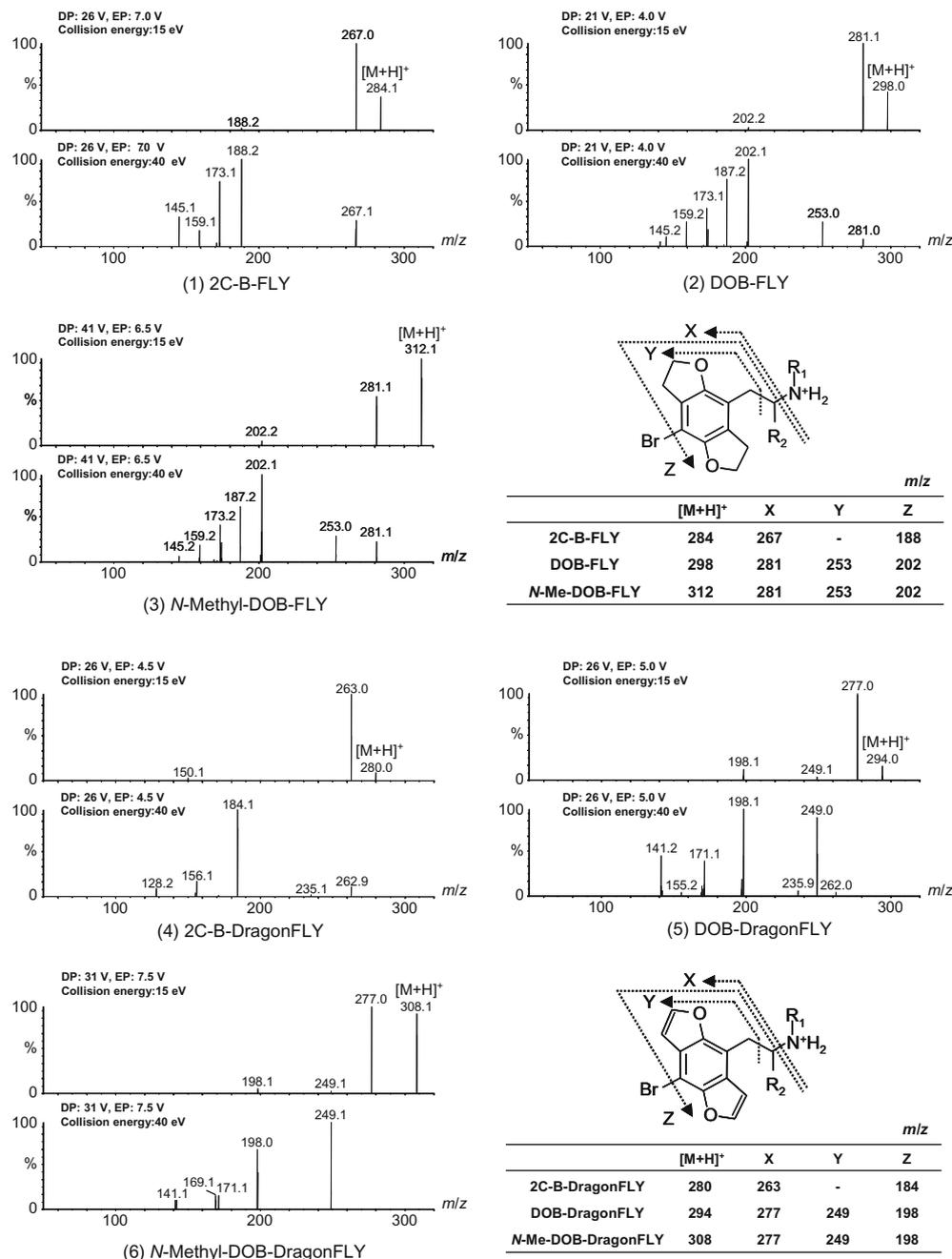


Fig. 7 Product ion spectra of the six compounds with different collision energies by LC-MS-MS and the schematic diagrams for their fragmentation modes



tifiable than ESI mass spectra [26,27]. LC-MS-MS was then explored for all analytes under the same chromatographic conditions as those used for LC-MS. Figure 7 shows product ion spectra of the analytes at two CEs and schematic diagrams for their fragmentation modes. As is observed for other phenethylamine-type drugs, elimination of an amino moiety was observed at lower CEs, followed by ethylene or bromine desorption at high CE (40 eV) [25,28]. The LODs of the analytes obtained by EPI (CE:15 eV) and SRM analyses are shown in Table 3. LC-MS-MS gave high sensitivity and specific product ion spectra for all analytes.

Conclusions

In this study, we synthesized six new hallucinogens classed as FLY and DragonFLY compounds, and conducted comprehensive analyses of them by GC-MS, LC-MS, and LC-MS-MS. Each method was found to have its advantages and disadvantages. Among the methods, GC-MS is the most inexpensive and most widely available throughout the world. We presented GC-MS methods for both free bases and TFA-derivatives of the compounds. Although TFA derivatization was useful for good mass spectral identification and sensitive quan-

Table 3 Limits of detection for analytes measured by LC-MS-MS

| Compound | EPI ^a | SRM ^b |
|--------------------|------------------|------------------|
| 2C-B-FLY | 5 | 0.3 |
| DOB-FLY | 5 | 0.3 |
| N-Me-DOB-FLY | 5 | 0.1 |
| 2C-B-DragonFLY | 10 | 0.5 |
| DOB-DragonFLY | 5 | 0.5 |
| N-Me-DOB-DragonFLY | 5 | 0.1 |

EPI, Enhanced product ion scan; SRM, selected reaction monitoring

^aLowest concentration detectable with a clear product ion spectra. Data given in units of ng/ml

^bLowest concentration detectable with a signal-to-noise ratio of 3. Data given in units of ng/ml

titation, it should be noted that small amounts of FLY compounds are converted into DragonFLY compounds during TFA derivatization even in the presence of TEA.

Using LC-MS, we optimized the LC conditions; a phenyl-type column was useful for good separation of the six compounds. Using the column, LC-MS-MS analysis was examined for the compounds; product ion mass spectra at different CEs were presented, and the sensitivities by SRM were also examined. In comparison of GC-MS, LC-MS, and LC-MS-MS for analysis of the compounds, LC-MS-MS seems to give the most identifiable mass spectra and high sensitivity for quantitation, although LC-MS-MS instruments are very expensive and require costly maintenance. The analysis method can be selected according to laboratory circumstances and instrument availability for analysis of FLY and/or DragonFLY compound(s).

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