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Identification of a Suspicious Drug by Using Spectroscopic Techniques: A Forensic Analytical Chemistry Project

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ABSTRACT We identified a drug analog by using screening and confirmatory tests. Total ion chromatogram showed a major peak with a molecular ion of 190 m/z, but no mass spectrum match from the NIST library. A minor peak was identified as 1-benzylpiperazine (molecular ion = 176 m/z). Molecular ions of both peaks differed by 14 m/z units, suggesting a $-CH_2-$ group. Both peaks had the same base peak of 91 m/z. Derivatizing the drug analog with trifluoroacetic anhydride confirmed the presence of 1-benzylpiperazine. No reaction occurred with the major peak. We proposed a benzyl-4-methylpiperazine structure, which was confirmed by NMR studies.

KEYWORDS 1-benzyl-4-methylpiperazine, drug analogs, forensic chemistry, spectroscopy

INTRODUCTION

The Internet abounds in synthetic drugs that are acclaimed to have the same desired effects on the user as regulated drugs. The misconception is that these drug analogs are chemically different from the regulated drugs and thus provide a legal and harmless version of the illegal parent narcotics while generating similar desired effects. To protect consumers, the Controlled Substance Analog Act of 1986 enacted regulatory laws that deal with possession and use of designer drugs.[1] Essentially, this act states that possession with the intent to consume any psychoactive substance that has a chemical structure that is substantially similar to that of a schedule I or II substance is in violation of the law. A short list of names of drug analogs that we obtained through conversation with the Georgia Bureau of Investigation (GBI) staff included sage, crystal, mushroom 2, cloud 9, trip2nite, and—more recently—k2 and bath salt. Crime labs and law enforcement organizations across the world are faced with a daunting task of identifying these recreational drugs because their chemical composition is ever changing. Unfortunately for the drug user, some of these drugs bring about undesirable health issues. In order to educate the community about harmful drugs, knowledge of the chemical composition of such drugs is paramount. The

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same knowledge gives law enforcement organizations some leverage in protecting the community by regulating harmful drugs. In academia, a need to provide relevant context for undergraduate students taking forensic analytical chemistry and instrumental analysis chemistry classes can be addressed by analyzing drug analogs that are in current use.[2,3] There are several advantages to the student in the instructor's using such current events to teach forensic chemistry. The student (investigator) is encouraged to obtain high-quality data and accurate interpretation, since the final results will be used to make a ruling on the suspect.[4] There is a high level of anxiety and motivation to determine the chemical nature of the drug analog and draw conclusions about whether such a drug is indeed different from the regulated drug. The student becomes more familiar with structural interpretation using spectroscopy and other techniques. Ultraviolet/Visible (UV/VIS), attenuated total reflection/fourier transform infrared (ATR/FTIR), gas chromatography combined with mass spectrometer (GC/MS), and nuclear magnetic resonance (NMR) instruments are readily available in undergraduate laboratories nowadays. These instruments provide useful qualitative information in analytical spectroscopy that depends on how matter (drugs) interacts with electromagnetic radiation. Qualitative information is the basis for identification of physical evidence in forensic investigation. Noting that the depth of information provided by these instruments differs, the Scientific Working Group on Drug Analysis (SWGDRUG) has classified analytical methods according to the depth of information they provide.[5] According to this group, FTIR, mass spectrometry, and NMR are placed in category A with maximum potential discriminative power, while UV/VIS is placed in category C. Uses of mass spectrometry coupled with GC cover a wide range of applications, including identification of heroin in illicit preparations[6] and determination of illicit drugs in human hair.[7] Application of GC/MS in investigating arson cases by Dolan[8] is noteworthy. The use of FTIR to identify illicit drugs abounds in the literature.[9,10] In recent years, it has been shown that the discriminating power of infrared spectroscopy can be enhanced if coupled with multivariate analysis techniques.[11–13] We use these instruments to provide open-ended projects to students as part of their research experience before they graduate.

In the current study, we analyzed a synthetic drug whose chemical name on the container was "6-triflouro-N-benzyl-methyl piperazine.hcl" (TBMP). This name was suggestive of a class of numerous synthetic designer drugs that are piperazine derivatives.[14–16] Examples of this class include benzylpiperazine (BZP) and 1-(3-trifluoromethyl- piperazine (TFMPP). Both drugs are harmful to the extent that in 2002 the U.S. Drug Enforcement Administration placed them on schedule I of the Controlled Substance Act.[17] The aim of this study was to use a current situation to provide a learning experience for our forensic chemistry students by having them characterize the chemical structure of TBMP. Students become more motivated when the subject matter is current, and they can relate to it. Further, the significance of this study lies in the new chemical information about TBMP and its disseminating through the community of scientists. Our first challenge was the absence of chemical information about TBMP in the literature, based on the available search engines (SciFinder, Google.Com). For example, we could not find a good match of the mass spectrum, UV spectrum, or infrared spectrum of TBMP in the literature. This also meant that there was no specific method in the literature for analyzing TBMP. Thus, instead of following standard methods as provided by the American Society for Testing and Materials (ASTM), we used multiple but independent techniques, starting with presumptive tests (color tests, TLC, pH measurements) and then employing more discriminative techniques. This approach provided flexibility in the identification of TBMP and more learning experience for the student. The pH measurement gave us some idea about whether we were dealing with an acidic, neutral, or basic molecule. We then used ultraviolet and midinfrared spectroscopy to obtain general profile of this drug. We finally used GC/MS and NMR for confirmatory studies, in accordance with the general protocol used in forensic chemistry identification of physical evidence.

MATERIALS AND METHODS

Instrumentation was as follows: Shimadzu QP-5000 GC/MS (Shimadzu, USA), with electron ionization energy of 70 eV, was used for acquiring all spectra. The GC conditions were as follows: A DB5 capillary column with a 0.25 mm internal

diameter and 30 m length was used; injection port temperature was set at 250°C; helium carrier gas flow was 1 mL/min; oven temperature parameters included an initial temperature of 60°C maintained for 1.00 min followed by a temperature gradient of 25°C/min to 259°C, maintained for 4.0 min for a total run time of 13 min. For the derivatized samples, we changed the temperature gradient program to 15°C/min in order to maximize separation. Split mode injection at a ratio of 1:50 was used. Transfer line temperature between the GC and MS was set at 280°C. A DPX 300 Bruker NMR instrument (Bruker, USA) was used to acquire the H-NMR and ^{13}C-NMR spectra. For H-NMR, an average of 16 scans was recorded, while 128 scans were acquired and averaged for the ^{13}C-NMR spectra.

Procedure was as follows: For pH measurement, about 20 mg of powder were dissolved in 5 mL of deionized water by vortexing for 2 min in a I0 mL test tube. The pH of deionized water was read before and after the drug powder was added. Test kits were purchased from Public Safety, Inc. Following the narcotic identification kits (NIK) instructions, about 10 mg portions of the powder were tested for heroin, morphine, and codeine using test kit B, which contains concentrated nitric acid. Marijuana, hashish, and THC were tested for by using test kit E. This reagent contains 2% vanillin and 1% acetaldehyde in alcohol, chloroform, and concentrated hydrochloric acid. We tested for opiates by using test kit K. This reagent contains a 2% formaldehyde solution in concentrated sulfuric acid. Cocaine HCL and cocaine base were tested for by using test kit G, which contains a 2% cobalt thiocyanate dissolved in 1:1 water/glycerin, concentrated hydrochloric acid, and chloroform. Procedures for preparing these spot test solutions are available in forensic textbooks and literature as an alternative to buying test kits.[18,19] For TLC about 20 mg of sample were vortexed in 0.50 mL methanol, and the supernatant was spotted on a silica gel plate. Among several mobile phases tested, a solvent mixture of 4 mL ethyl acetate and 0.40 mL methanol gave good separation as opposed to smears. For UV spectral measurement, about 10 mg powder was vortexed in 4 mL methanol. The supernatant, after centrifuging at 2000 rpm for 10 min, was used to measure the UV spectrum using a Cary 4000 UV/vis Spectrometer (Varian, USA) against a methanol blank. The infrared spectrum was measured directly by the Perkin Elmer ATR/FTIR Spectrometer (USA) on the dry methanol extract. For GC/MS analysis, about 0.2 g TBMP powder was vortexed for 2 min in 4 mL solvent (water, methanol, acetone, and ethyl acetate), and the mixture was filtered through a 0.45 µm syringe filter. The clear solution was extracted with 3 × 1 mL hexane in the case of water. The methanol, acetone, and ethyl acetate extracts were preconcentrated by evaporation under a stream of nitrogen under the hood. Extractions were performed at pH 3 and 9 to establish if pH played any role on the efficiency of extraction. The pH was adjusted using 0.2 M solutions of HCI and NaOH. The prepared sample was finally injected into the GC/MS using 1 µL injection volumes. For NMR analysis, about 20–30 mg of drug powder were dissolved in 0.8 mL $CDC1_3$ in the NMR capillary tubing. This solution was used for both H-NMR and ^{13}C-NMR.

RESULTS AND DISCUSSION

The roughly 20 mg powder changed the pH of deionized water from pH 6 to 5, indicating slight acidity of the sample. Presumptive color tests were all negative. For test kit G, a test for cocaine, a pink coloration was observed instead of a blue positive color for cocaine. The TLC experiment showed one single separation observable under a UV lamp, implying that this product had one component as shown by the insert in Fig. 1. The UV spectrum showed maximum absorbance around 220 and 258 nm, a typical absorption of a substituted aromatic ring. The ATR/FTIR spectrum measured directly on the dried methanol extract, showed medium CH stretching at 2950–2847 cm^{-1} and strong overtones of substituted aromatic rings around 825–705 cm^{-1}. We could not find spectra in the literature that compared with our results.

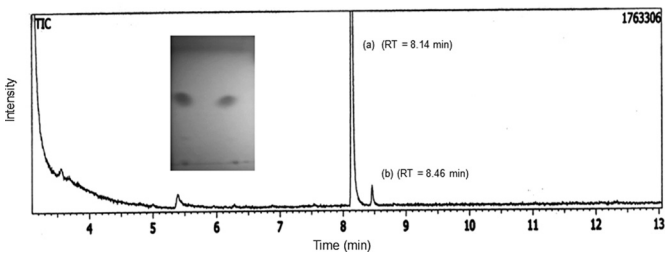

FIGURE 1 **Chromatogram of TBMP in ethyl acetate under basic conditions. The major and minor peaks at 8.14 and 8.46 min are in the ratio of 64 to 1. The insert is the TLC showing one component.**

For GC/MS analysis, extracts that gave well-defined total ion peaks were those by ethyl acetate around pH 9. Figure 1 shows a major peak at 8.14 min and a minor one at 8.46 min. The two peaks are in the ratio of 64 to 1, explaining why the TLC experiment did not detect the minor component (Fig. 1, insert). For peak a in Fig. 1, electron ionization (El) measurements using a GC/MS showed fragments at m/z = 42, 56, 70, 91, 99, 119, 132, 146, and 190. For peak b, intense fragments at m/z = 42, 56, 91, 134, and 176 and minor ones at m/z = 118, 120, and 146 were observed Fig. 2. Both peaks in Fig. 1 have large intensities at 91 m/z, with 96% and 100% abundances. The 91 m/z intensity indicates the presence of a benzyl ion that rearranges to a stable tropylium cation. Assuming 190 and 176 m/z (Fig. 2) to be molecular ions of peaks a and b in Fig. 2, the even m/z intensities suggest an even number of N atoms in both molecules.

While the larger peak was not directly identified from the NIST Library, the smaller peak was easily identified by El-MS as 1-benzylpiperazine (BZP), and its m/z fragments at 42, 56, 91, 134, and 176 have been reported by Wikstrom and coworkers.[20] Based on the suggested molecular ion of 190 m/z for the compound in Fig. 2a, we proposed that the difference in mass units of 14 m/z was due to a -CH_3 substituted to the benzyl ring Fig. 3, Structure 1

Further, the isotopic intensities of 190 m/z (20.4%) and 191 m/z (2.8%) estimate the number of C atoms in this molecule to be 12. For further structural elucidation, a dry extract of the TBMP tablet was derivatized by incubation in 100 µL ethyl acetate and I00 µL trifluoroacetic anhydride (TFAA) at 65–70°C for 45 min in a sand bath. To maximize separation,

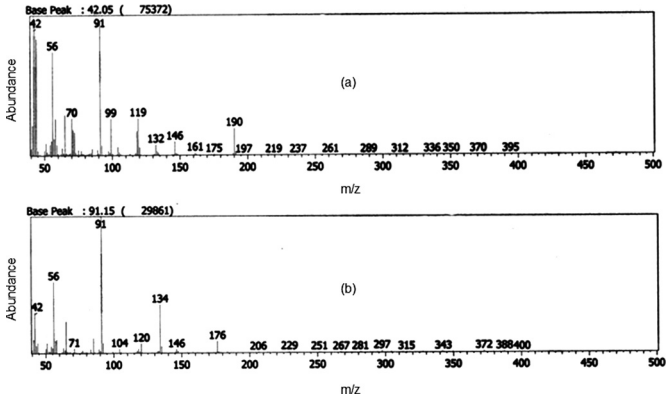

FIGURE 2 Mass spectrum of (a) the major peak showing a molecular ion of 190.25 amu and (b) the minor peak showing a molecular ion of 176.25 amu.

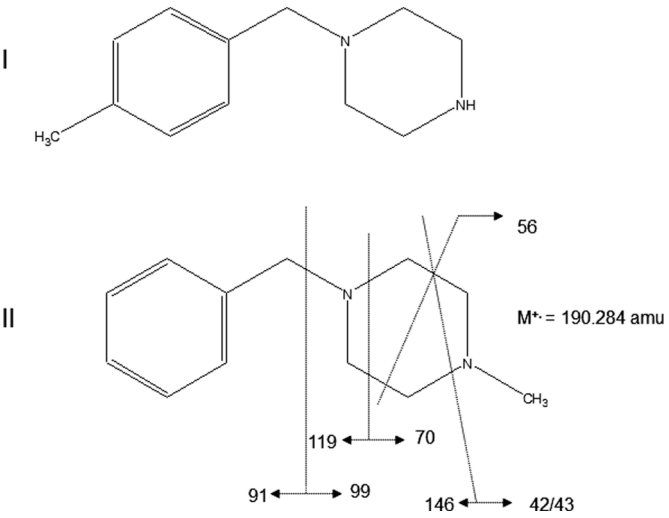

FIGURE 3 Structure I proposed for the major peak before derivatization. Structure II proposed after derivatization, showing that the major peak is 1-benzyl-4-methylpiperazine instead of 4-methylbenzylpiperazine.

we used a temperature gradient of 15°C/min between 60 and 259°C. The two peaks were separated at 12.86 min and 14.50 min as shown by the chromatogram top of Fig. 4.

The mass spectrum of peak a did not change from that obtained prior to derivatization, an indication that compound a, middle of Fig. 4, did not react with TFAA. Compound b, bottom of Fig. 4, reacted with TFAA by replacement of H-atom in the piperazine moiety, giving intensities at m/z = 43, 57, 91,146, 181, 195, and 272, with 272 m/z as the molecular

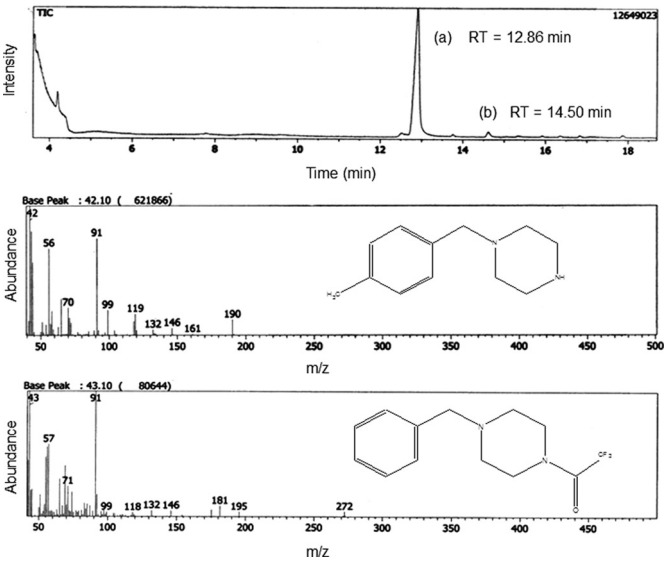

FIGURE 4 Top: TBMP chromatogram after derivatization with TFAA showing (a) the major peak and (b) the minor peak. Middle: The spectrum of the major peak is unchanged. Bottom: The minor peak, 1-benzylpiperazine reacted with TFAA.

ion. This pattern of m/z fragments was also reported for TFAA-derivatized benzylpiperazine.[14] The observation that compound a did not react with TFAA indicates that the methyl group in compound a is not on the benzyl ring as we first proposed, but it is on the>N-H end of the piperazine moiety. Thus when -CH_3 replaces the H-atom, it prevents this molecule from reacting with TFAA. In GC/MS experiments, derivatizing a molecule is often used to make the molecule thermally stable and to improve peak shape. In this instance, derivatization of TBMP enabled us to propose a better structure. Thus in Fig. 3, Structure II is the preferred one compared to Structure I. Figure 3 also shows possible fragments of Structure II leading to the observed intensities. The name of this compound, via ChemDraw software, is "1-benzyl-4-methylpiperazine." A compound with identical fragment patterns has been reported recently by Takahashi and coworkers[21] in their efforts to build a designer-drugs data library. This compound is claimed to have similar effects as those of BZP, but its stimulant effect is slightly weaker, and it seems to have less negative side effects such as headaches and nausea.[22]

H-NMR and ^{13}C-NMR analysis is as follows: Fig. 5 is H-NMR spectrum obtained directly on the TBMP tablet in $CDCl_3$ as a solvent. This spectrum shows five distinct groups of H- atoms: five protons downfield belonging to the aromatic ring ($\delta = 7.3$ ppm), two protons ($\delta = 3.6$ ppm) due to the benzyl -CH_2- between the aromatic ring and the first N-atom of the piperazine moiety, and four protons ($\delta = 3.1$ ppm)

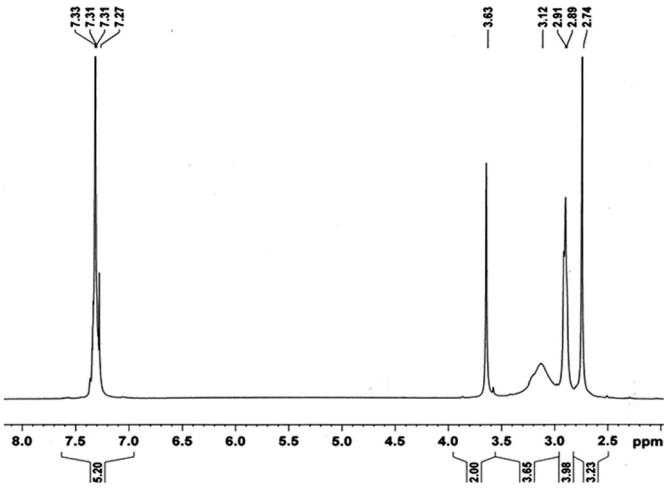

FIGURE 5 H-NMR of TBMP, showing five groups of H-atoms (see text), thus confirming Structure II in Fig. 3.

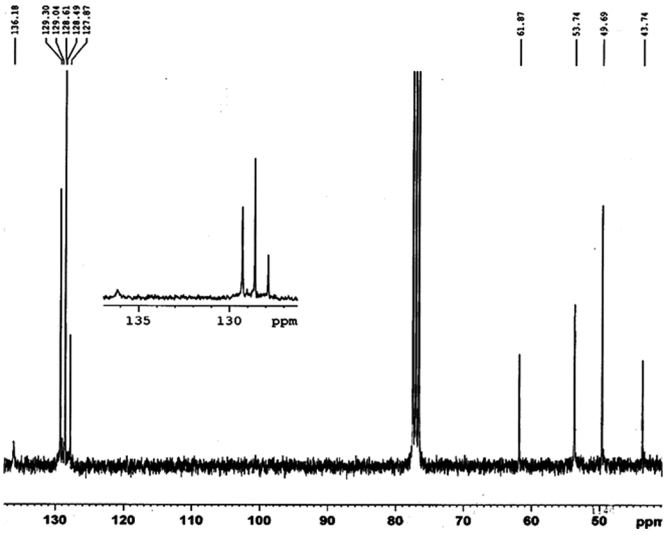

FIGURE 6 13C-NMR of TBMP, showing five groups of C-atoms (see text), thus confirming Structure II in Fig. 3.

due to the two -CH_2- groups next to the first piperazine moiety. Close to this group are four protons ($\delta = 2.9$ ppm) due to the two -CH_2- next to the>N-CH_3 end of the piperazine moiety. The most up-field intensity with three protons ($\delta = 2.7$ ppm) is due to -CH_3 for the proposed structure II in Fig. 3. The ^{13}C-NMR spectrum Fig. 6 indicates five groups of C-atoms: the aromatic ring group around 130 ppm, the -CH_2- intensity at 61.87 ppm, two intensities at 53.7 and 49.69 ppm belonging to the piperazine moiety, and a methyl intensity at 43.74 ppm.

Both nmr spectra support the structure displayed in Fig. 3(II). This molecule differs in mass from benzyl piperazine (m/z = 176) by 14 mass units, well accounting for m/z = 190 for 1-benzyl-4-methylpiperazine. It is possible that 1-benzyl-4-methylpiperazine was synthesized from benzylpiperazine, which would account for the minor peak of benzylpiperazine in Fig. 1 as a residual. We have not been able to link structure II in Fig. 3 to the name label "6-triflouro-N-benzyl-methyl piperazine.hcl " on the container. The CAS number from Wikipedia encyclopedia[22] is 374898-00-7, and its IUPAC name is 1-benzyl-4-methylpiperazine, the same name that we obtained from the ChemDraw software. To date, this compound is not listed in the SWGDRUG library or SciFinder®

CONCLUSION

The drug TBMP contains one major component and a minor one. Based on GC/MS and NMR spectra

analysis, the major component in TBMP is 1-benzyl-4-methylpiperazine (Fig. 3, II), and the minor component is 1-benzyl piperazine. This drug is soluble in water and methanol. It was not positive with any of the color tests we used. A ratio of 64:1 for 1-benzyl-4-methylpiperazine to 1-benzyl piperazine indicates that 1-benzyl-4-methylpiperazine was probably prepared from 1-benzyl piperazine. To date there is very little reported in the literature about 1-benzyl-4-methylpiperazine in comparison to 1-benzylpiperazine. For the student, this project provides a solid strategy in forensic chemistry for identifying drugs, starting with presumptive tests (color, pH, TLC, UV, IR) and ending with confirmation tests (GC/MS, NMR). Students learn how to use analytical spectroscopy to predict the chemical structure of a molecule.

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