Synthesis and Characterization of Benzoylfentanyl and Benzoylbenzylfentanyl

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ABSTRACT: The synthesis and characterization of benzoylfentanyl and benzoylbenzylfentanyl via gas chromatography-mass spectrometry, Fourier transform infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy were conducted to confirm the identification of two seized exhibits.

KEYWORDS: Fentanyls, Fentanyl-Related Compounds, Illicit Drugs, Analysis, Forensic Chemistry.

Introduction

As of December 2017, fentanyl and 29 other fentanyl-related compounds (FRCs) have been identified in DEA casework [1]. Only a limited number of these compounds have been reported in the literature, however [1-5]. The syntheses and characterization of two new FRCs, benzoylfentanyl and benzoylbenzylfentanyl, are presented herein (Figures 1a,b). At this laboratory

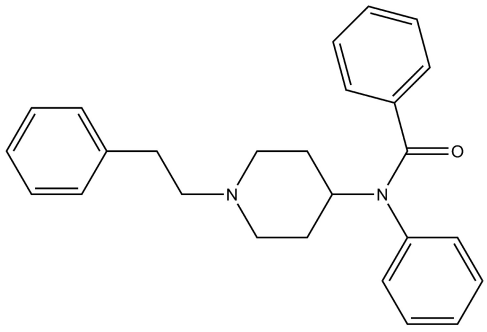

Figure 1a. Benzoylfentanyl
[$C_{26}H_{28}N_2O$; mw = 384.5]

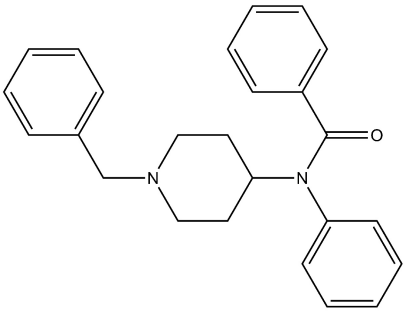

Figure 1b. Benzoylbenzylfentanyl
[$C_{25}H_{26}N_2O$; mw = 370.5]

(STRL), the structures of FRCs are typically confirmed by proton nuclear magnetic resonance spectroscopy (1H-NMR); however, the majority of forensic laboratories do not have NMR spectrometers, and therefore rely on gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR) for the identification of drug exhibits. In many cases, however, GC-MS and/or FTIR do not produce unambiguous identification and differentiation of closely related compounds; therefore, standards are needed for direct comparisons of retention data and spectra. Herein, we report the syntheses of benzoylfentanyl and benzoylbenzylfentanyl and their characterization by GC-MS, FTIR, and 1H-NMR. The GC-MS results allow for the unambiguous identification of these two FRCs in sample analysis.

Experimental

Chemicals, Reagents, and Materials
All solvents (except the NMR solvents) were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). NMR solvents were from Cambridge Isotopes (Tewksbury, MA). All other chemicals were reagent-grade quality and products of Sigma-Aldrich Corporation (Milwaukee, WI).

Synthesis of Benzoylfentanyl and Benzoylbenzylfentanyl
Both syntheses were conducted at this laboratory. In accordance with *Journal* policy, exact experimental details are not provided, but are outlined in Figures 2 and 3 (see next page). Briefly, 4-ANPP was reacted with benzoyl chloride to give benzoylfentanyl, which was converted to its HCl ion-pair in 80% overall yield. 1-Benzyl-4-piperidone was reacted with aniline to give 1-benzyl-4-anilinopiperidone, which was then reacted with benzoyl chloride to give benzoylbenzylfentanyl, which in turn was converted to its HCl ion-pair in 37% overall yield.

Gas Chromatography-Mass Spectrometry
Mass spectra were obtained on two different instruments, as detailed below. GC-MS Method #1 was used at this laboratory for the analyses of the benzoylbenzylfentanyl exhibit and reference standard, while GC-MS Method #2 was used at the Mid-Atlantic Laboratory for the analyses of the benzoylfentanyl exhibit and reference standard.

Method #1: Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC) (Palo Alto, CA). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5 : 1) at 280°C. The MSD source was operated at 230°C.

Method #2: Mass spectra were obtained on an Agilent Model 5977A quadrupole MSD that was interfaced with an Agilent Model 7890B GC. The MSD was operated in the EI mode with an ionization potential of 70 eV, a scan range of 40-500 amu, and a scan rate of 3.2 scans/s. The GC was fitted with a 15 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-5MSUI (J & W Scientific). The oven temperature was programmed as follows: Initial temperature, 145°C; initial hold,

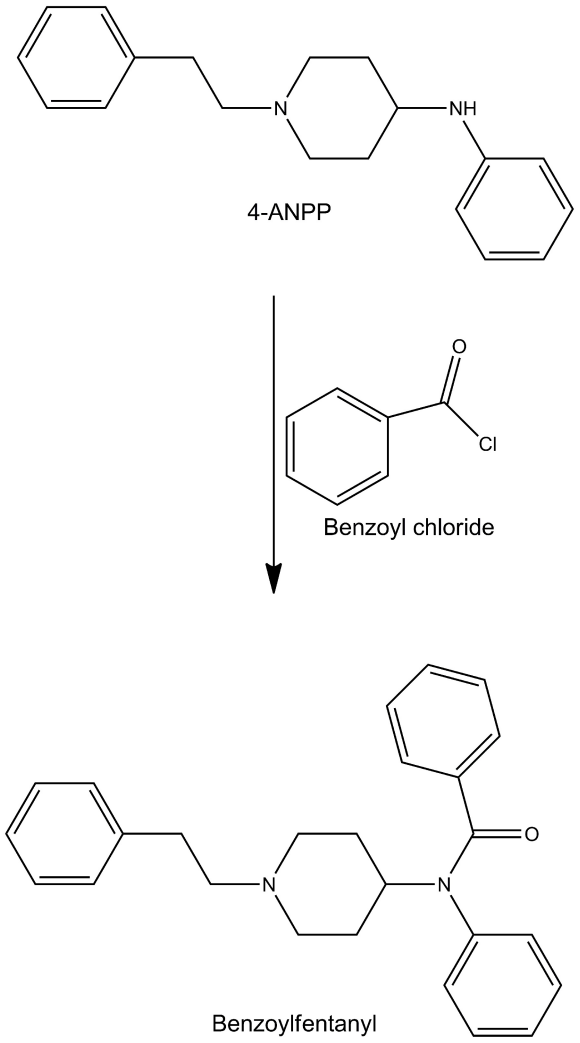

Figure 2. Synthesis of Benzoylfentanyl.

1.0 min; program rate, 20ºC/min to 280ºC, hold for 0.25 min; program rate, 45ºC/min to 295˚C, hold for 3.167 min. The injector was operated in the split mode (20 : 1) at 280ºC. The MSD source was operated at 230ºC.

Fourier Transform Infrared Spectroscopy / Attenuated Total Reflectance

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory (Madison, Wisconsin). Instrument parameters were: Resolution = 4 cm^{-1}; gain = 8;

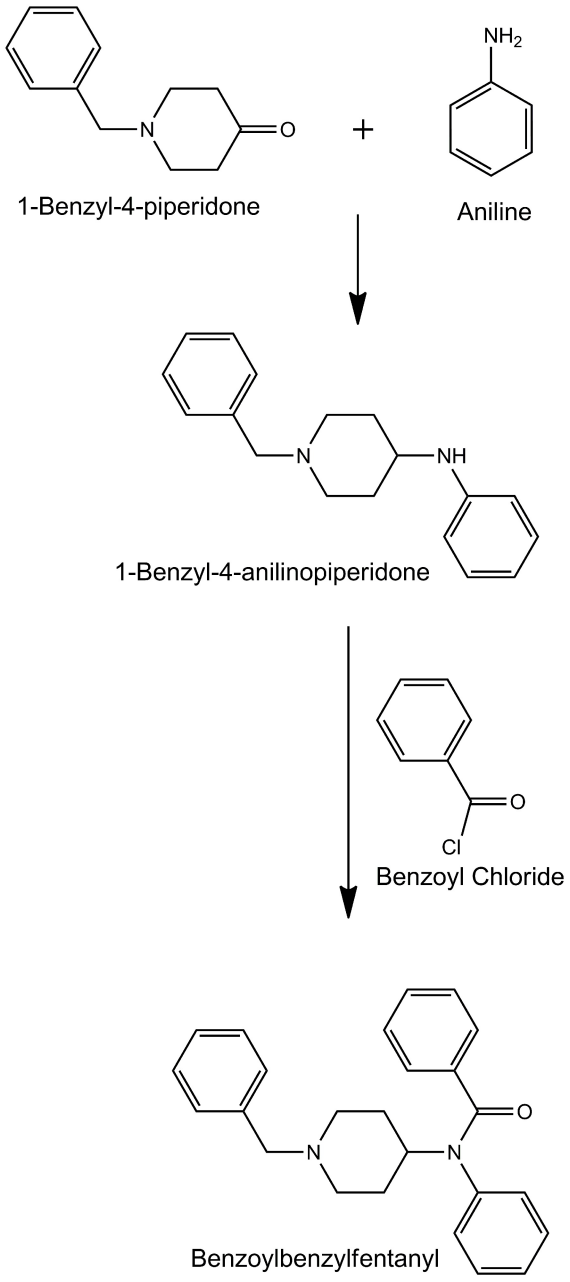

Figure 3. Synthesis of Benzoylbenzylfentanyl.

optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Proton Nuclear Magnetic Resonance Spectroscopy

1H-NMR spectra were obtained on an Agilent 600MR-DD2 600 MHz NMR equipped with a 5

mm OneNMR pulse field gradient probe (Palo Alto, CA). The sample temperature was maintained at 25°C. Standard Agilent pulse sequences were used to obtain proton, carbon-13 (proton decoupled), HSQC, HMBC (C13 and N15), COSY, H2BC, and NOESY spectra for structural elucidation (however, only the proton spectra are presented herein). Samples were dissolved in 1 mL deuterated chloroform ($CDCl_3$) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference.

Results and Discussion

Case #1 (approximately 36 grams of white powder seized in West Virginia in July, 2017; exact location and details sensitive): The exhibit was submitted to the DEA Mid-Atlantic Laboratory. GC-MS (Method #2) analysis (Figure 4, upper) identified benzoic acid, α-PVP, caffeine, U-47700, alprazolam, and an unknown, late-eluting compound at approximately 14.6 minutes. The unknown produced a mass spectrum with a base peak at *m/z* 105 and other major ions at *m/z* 77, 197, and 293 (Figure 5, next page, upper). The apparent molecular ion (i.e., M-2H) was at *m/z* 382. The unknown was therefore suspected to be benzoylfentanyl based on the fragment ions and the late retention time. The Mid-Atlantic Laboratory requested that the Fentanyl Signature Profiling Program of this laboratory

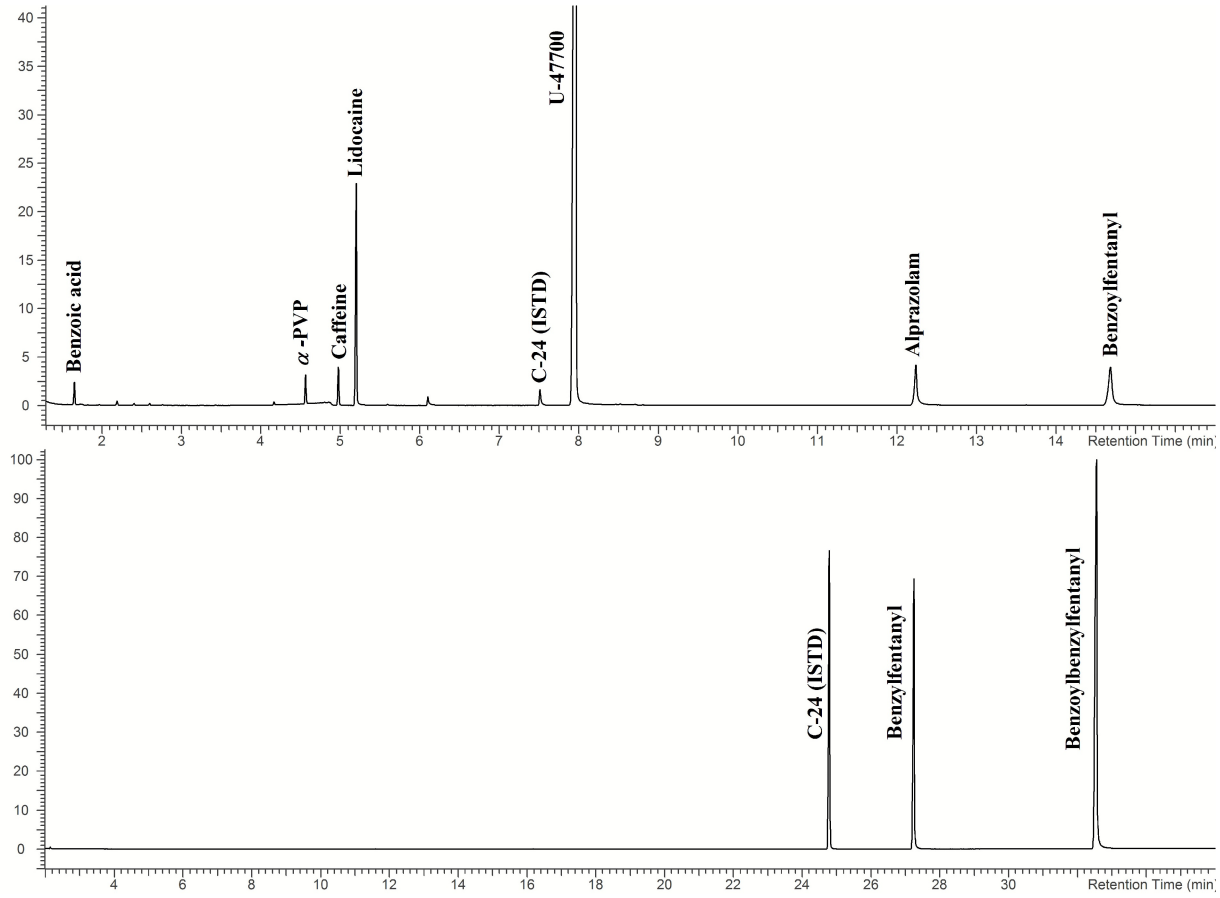

Figure 4. Reconstructed Ion Chromatograms of (Upper) Case #1 and (Lower) Case #2.

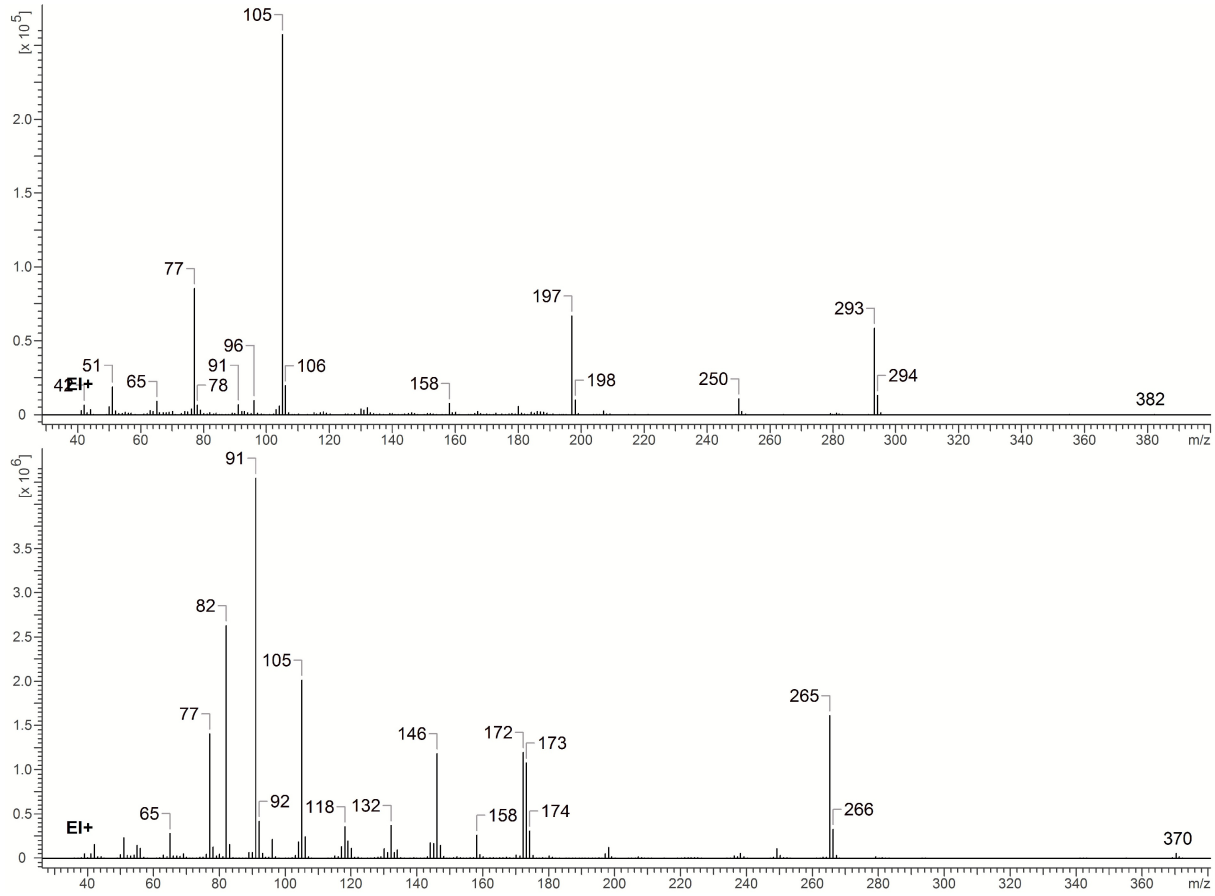

Figure 5. Mass Spectra (Upper) of Benzoylfentanyl and (Lower) Benzoylbenzylfentanyl.

(FSPP) synthesize a reference standard of this compound. GC-MS comparison of the synthesized reference material with the unknown peak gave an identical retention time and mass spectrum, thus confirming the identification. The concentration of benzoyl-fentanyl in the powder was determined to be approximately 1%.

Case #2 (approximately 48 grams of white powder seized in California in August, 2017; exact location and details sensitive): The exhibit was submitted to the DEA Western Laboratory. GC-MS (Method #1) analysis (Figure 4, lower) identified benzylfentanyl and an unknown, late-eluting compound at approximately 32.5 minutes. The unknown produced a mass spectrum with a base peak at *m/z* 91 and other major ions at *m/z* 77, 82, 105, 146, 172, and 265 (Figure 5, lower). The apparent molecular ion was at *m/z* 370. The unknown was therefore suspected to be benzoylbenzylfentanyl based on the fragment ions and the late retention time. The Western Laboratory requested that the FSPP synthesize a reference standard of this compound. GC-MS comparison of the synthesized reference material with the unknown peak gave an identical retention time and mass spectrum, thus confirming the identification. The concentration of benzoylbenzylfentanyl in the powder was determined to be approximately 64%.

The FTIR/ATR and 1H-NMR spectra for the benzoylfentanyl HCl and benzoylbenzylfentanyl HCl reference standards, as acquired at this laboratory, are illustrated in Figures 6 and 7, respectively (next two pages); Figure 7 includes expansion plots. [The actual case exhibits were not submitted to FTIR or NMR analyses at this laboratory.]

Cautionary Note

The relative potencies of these two FRCs are unknown; for this reason, analysts should exercise appropriate care with exhibits suspected to contain either one of these compounds – or any other FRCs [6].

References

[1] Song K, de Armas AM. The analytical profile of fluorobutyryl fentanyl isomers. Abstracts, American Academy of Forensic Sciences 70th Annual Meeting, Seattle, Washington, February 19 - 24, 2018:B189. [Note: According to the Advanced Program, 15 presentations on fentanyl and/or fentanyl-related compounds will be made at this meeting; see: https://www.aafs.org/wp-content/uploads/CompleteAP.pdf Date of most recent access: January 24, 2018.]

[2] Vardanyan RS, Hruby VJ. Fentanyl-related compounds and derivatives: Current status and future prospects for pharmaceutical applications. Future Medicinal Chemistry 2014;6(4):385-412.

[3] United Nations Office on Drugs and Crime (UNODC). Global SMART Update. Fentanyl and its analogues – 50 years on. Posted at: http://www.unodc.org/documents/scientific/Global_SMART_Update_Vol.17_web.pdf. [Date of most recent access: January 10, 2018.]

[4] Noble C, Weihe Dalsgaard P, Stybe Johansen S, Linnet K. Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MSE mode and retrospective analysis of authentic forensic blood samples. Drug Testing and Analysis 2017: doi: 10.1002/dta.2263

[5] Kanamori T, Iwata YT, Segawa H, Yamamuro T, Kuwayama K, Tsujikawa K, Inoue H. Characterization and differentiation of geometric isomers of 3-methylfentanyl analogs by gas chromatograph/mass spectrometry, liquid chromatography/mass spectrometry, and nuclear magnetic resonance spectroscopy. Journal of Forensic Sciences 2017: doi: 10.1111/1556-4029.13395

[6] Fentanyl: A Briefing Guide for First Responders (2017). The following website includes multiple briefs on fentanyl and FRCs, including this First Responders Briefing Guide: https://www.dea.gov/druginfo/fentanyl.shtml [Date of most recent access: January 21, 2018.]

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[Figures 6 and 7 Follow.]

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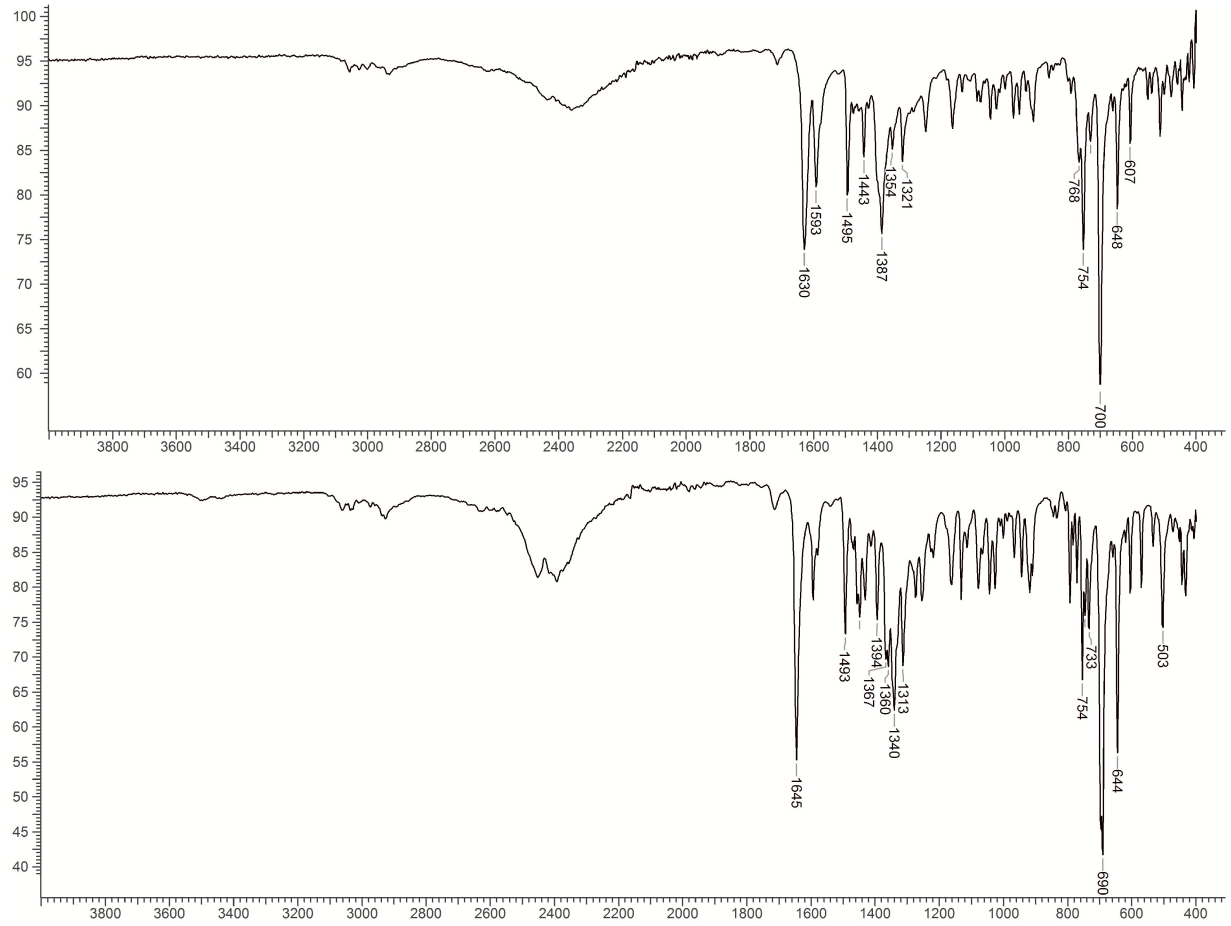

Figure 6. FTIR/ATR (Upper) of Benzoylfentanyl HCl and (Lower) of Benzoylbenzylfentanyl HCl.

[Figure 7 Follows.]

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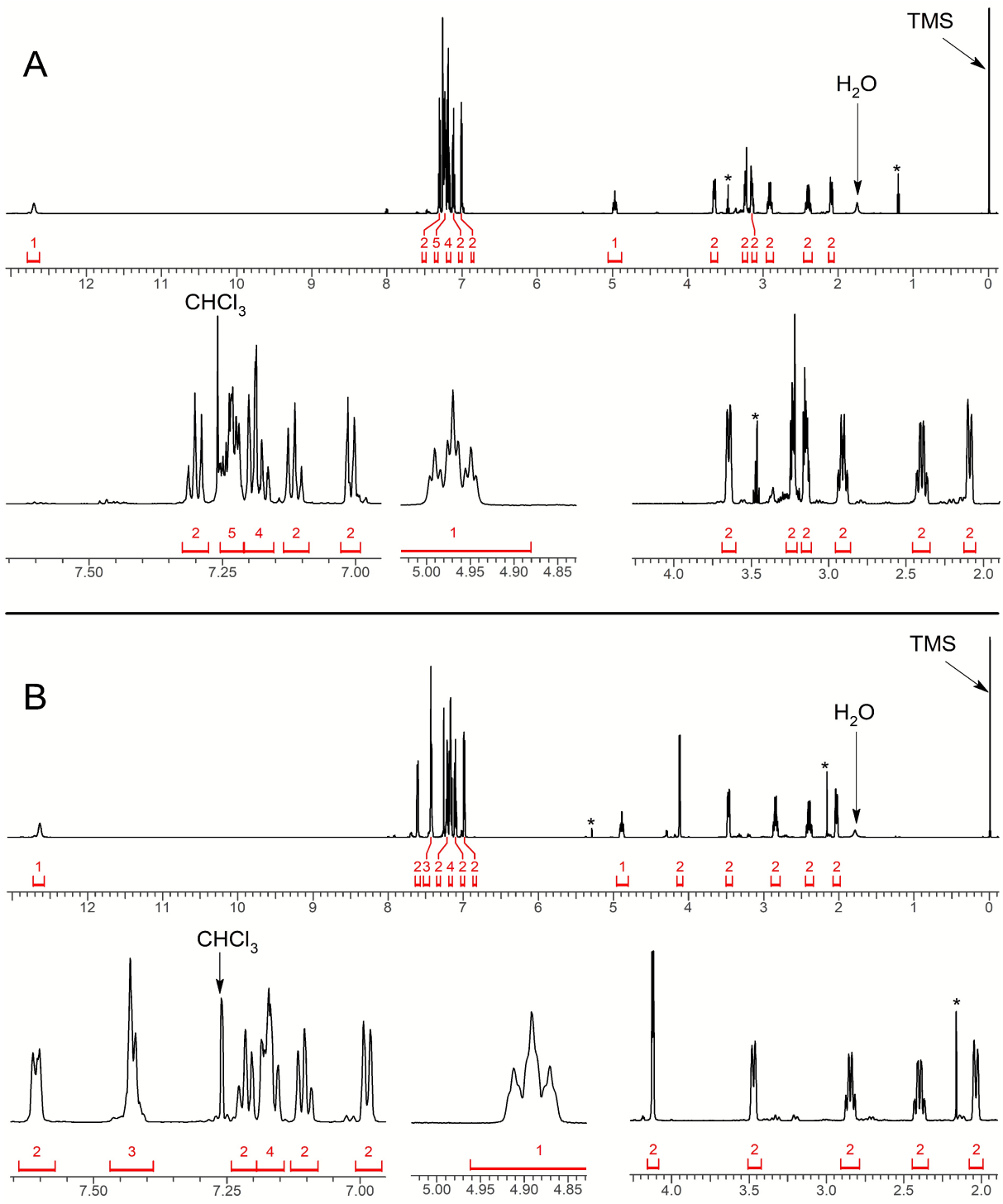

Figure 7. 1H NMR Spectra of (A) Benzoylfentanyl HCl and (B) Benzoylbenzylfentanyl HCl. [Note: Compound preparation solvent impurities are marked with an asterisk (*) above them.]