N-Acetylbenzocaine: Formation via Transacetylation of Benzocaine and Acetylsalicylic Acid in a Cocaine Exhibit

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ABSTRACT: N-Acetylbenzocaine was recently identified in an illicit cocaine HCl exhibit which also contained salicylic acid and traces of acetylsalicylic acid, and benzocaine. This paper discusses the analysis and characterization of N-acetylbenzocaine, as well as its transacetylation synthesis pathway. Supporting analytical data from gas chromatography/mass spectrometry, gas chromatography flame ionization detection, Fourier-transform infrared spectroscopy, and Fourier-transform nuclear magnetic resonance spectroscopy are presented.

KEYWORDS: N-acetylbenzocaine, transacetylation, synthesis, characterization, forensic chemistry

The DEA Western Laboratory recently received a white crystalline substance as a suspected cocaine exhibit. The exhibit was determined to contain 22.5% cocaine HCl, salicylic acid, and traces of acetylsalicylic acid (asprin) and benzocaine. The exhibit also contained a significant amount of an unidentified compound. The unknown compound produced a mass spectrum (Figure 1a) having similar ions to benzocaine (Figure 1b), with an apparent molecular ion at *m/z* 207. The presence of an ion at *m/z* 43 suggested that the compound may be N-acetylbenzocaine. A literature search revealed that N-acetylbenzocaine has not been reported previously in an illicit drug exhibit. However, N-acetylbenzocaine has been identified previously as a metabolite of benzocaine in fish and guinea pigs [1-5]. The unknown compound was determined to be N-acetylbenzocaine (Figure 2) after it was synthesized and fully characterized via gas chromatography/mass spectrometry (GC/MS), infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of reagent grade quality and products of Aldrich Chemical (Milwaukee, WI).

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass specta were obtained on an Agilent Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph (GC). The GC/MSD was operated under conditions similar to those reported previously [6].

Infrared (IR) Spectroscopy

Infrared spectra were obtained on a Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Experiments were performed at 25°C with a Varian 600 MHz VNMRS nuclear magnetic resonance (NMR) spectrometer using a 5 mm broadband Nalorac Z-Spec probe (Varian Inc., Palo Alto, CA). Standard vendor supplied experiments were used to obtain proton and carbon (proton decoupled) spectra, and gradient versions of the heteronuclear multiple bond correlation (gHMBC) and heteronuclear single quantum coherence (gHSQC) spectra.

Gas Chromatography/Flame Ionization Detection (GC/FID)

N-Acetylbenzocaine determination: GC/FID analyses were performed with an Agilent (Palo Alto, CA) Model 6890N gas chromatograph. The sample preparation and GC/FID were operated under the same conditions as those reported previously [7]. Isopropylcocaine was utilized as the internal standard and the unknown exhibit was bracketed with concentrations of 0.15 and 0.58 mg/mL of N-acetylbenzocaine (correlation coefficient, $R^2 = 0.9996$).

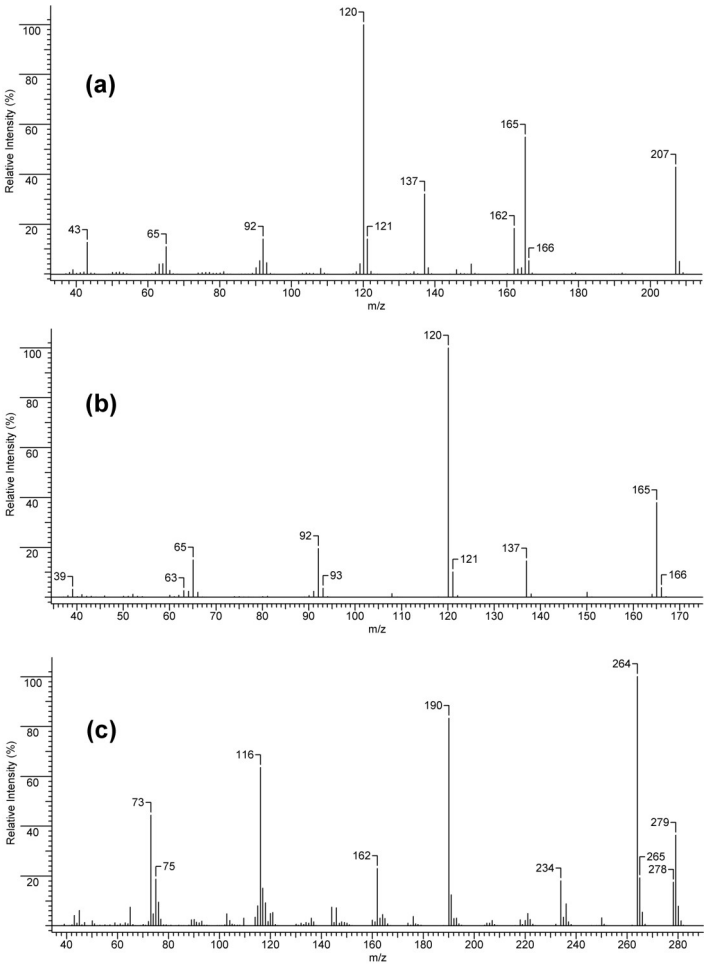

Figure 1 - Electron ionization mass spectra of (a) N-acetylbenzocaine, (b) benzocaine, and (c) N-acetyl-benzocaine-TMS derivative.

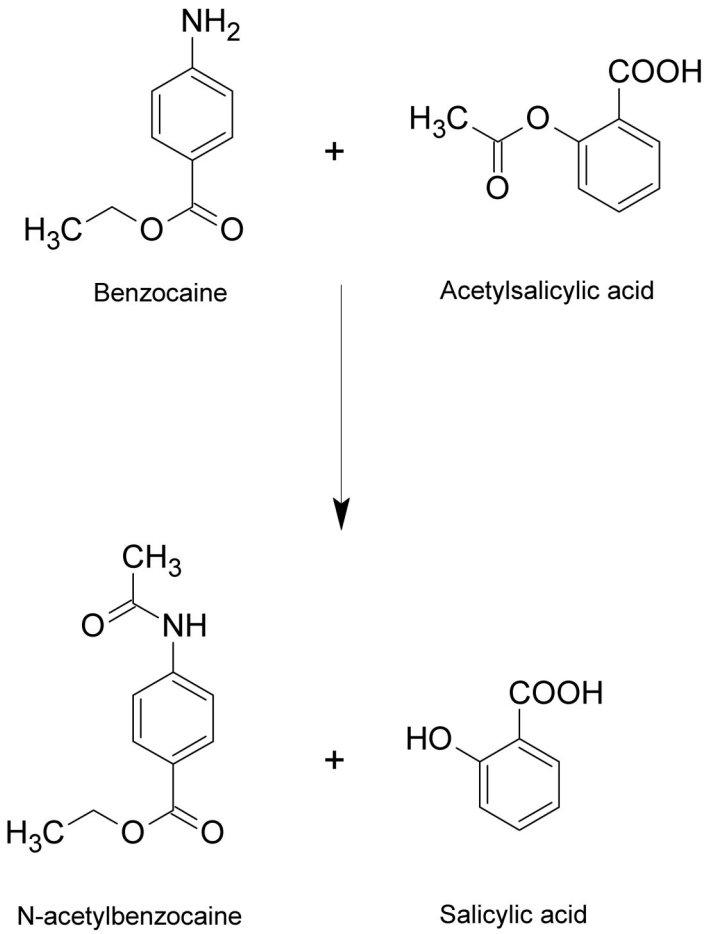

Figure 2 - Transacetylation of benzocaine and acetylsalicylic acid.

Synthesis

N-acetylbenzocaine: Benzocaine (243 mg, 1.47 mmol) was heated with 2.0 mL of acetic anhydride for 30 min. at 75°C in a sealed tube. The reaction was cooled and quenched with 10 mL of water, solid Na_2CO_3 was added until pH = 8, and the reaction was extracted with chloroform (2 x 10 mL). The extracts were combined, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to a white powder (251 mg, 99% purity, 82% yield).

Transacetylation of benzocaine and acetylsalicylic acid: Benzocaine (13 mg, 0.079 mmol) and acetylsalicylic acid (26 mg, 0.14 mmol) were heated (neat) overnight at 70°C. The resulting product was dissolved in chloroform, examined via GC/MS, and found to contain a significant amount of N-acetylbenzocaine (yield not calculated).

Results and Discussion

Examination of the reconstructed total ion chromatogram for the illicit exhibit (Figure 3a) determined that cocaine (Peak #7), and salicylic acid (Peak #1) were present, in addition to lesser amounts of acetylsalicylic acid (Peak #3) and benzocaine (Peak #4). The unknown compound (Peak #6) represented over 50% of the total ion current. Its mass spectrum (Figure 1a) produced an apparent molecular ion at *m/z* 207. The spectrum was markedly similar to benzocaine (Figure 1b), exhibiting several ions in common, thus suggesting a benzocaine derivative. The presence of an ion at *m/z* 43 and a mass difference of +42 Daltons (Da) from benzocaine suggested that the compound was N-acetylbenzocaine. The unknown compound did form a TMS derivative (Figure 3b producing Figure 1c), indicating one labile proton within the molecule and consistent with a molecular weight of 207 for the underivatized moiety. The direct infrared spectrum of the exhibit (Figure 4a) indicated a significant amount of an amide present due to an apparent amide carbonyl stretch at *ca.* 1680 cm^{-1} and an N-H stretch at *ca.* 3330 cm^{-1}.

A reference standard of N-acetylbenzocaine was synthesized and its mass spectrum and retention time were compared to the unknown compound, both derivatized and underivatized. The unknown was identical in all respects and was identified as N-acetylbenzocaine. The NMR spectrum (Figure 5), chemical shifts (Table 1), and IR (Figure 4b) of the synthesized standard are also presented. Finally, GC/FID analysis determined N-acetylbenzocaine to be 35.2% of the illicit exhibit.

N-acetylbenzocaine is not a commercially available product and the possibility that it was intentionally added as an adulterant is highly unlikely. Since salicylic acid and traces of benzocaine and acetylsalicylic acid were also present, we postulated that N-acetylbenzocaine may have arisen from transacetylation of benzocaine and acetylsalicylic. Acetylsalicylic acid (aspirin) is known to transacetylate human proteins *in vivo* [8] as well as transacetylate acetaminophen to its acetyl derivative [9]. The

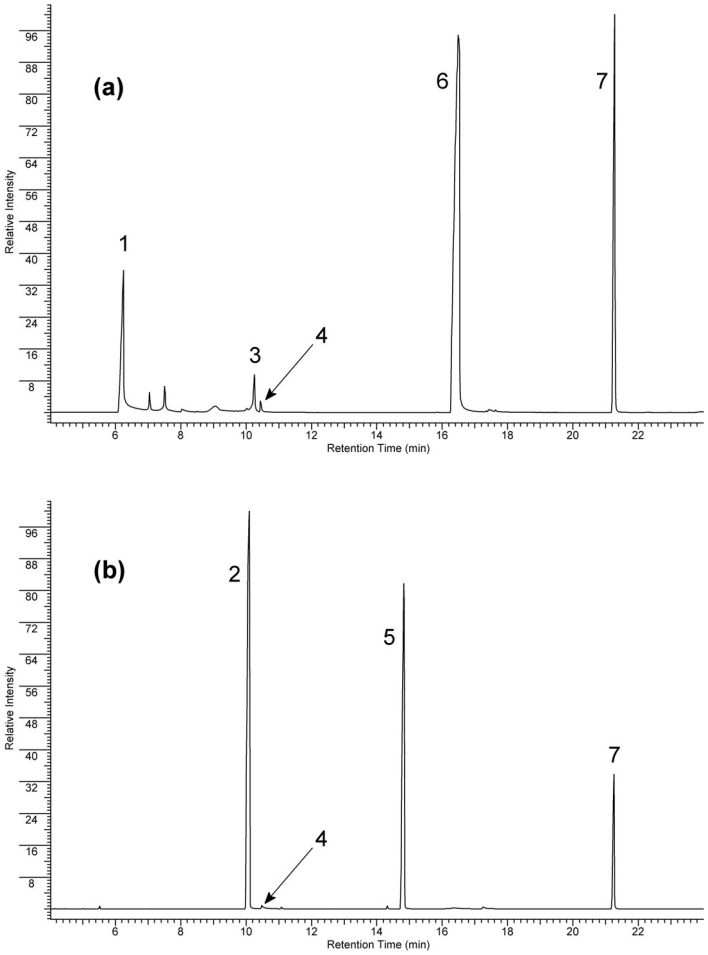

Figure 3 - Partial reconstructed total ion chromatogram of a 22.5% cocaine exhibit containing 35.2% N-acetylbenzocaine (a) underivatized and (b) derivatized with MSTFA. Peak identification: 1 = salicylic acid, 2 = salicylic acid-di-TMS derivative + trace acetylsalicylic acid, 3 = acetylsalicylic acid, 4 = benzocaine, 5 = N-acetylbenzocaine-TMS derivative, 6 = N-acetylbenzocaine, and 7 = cocaine.

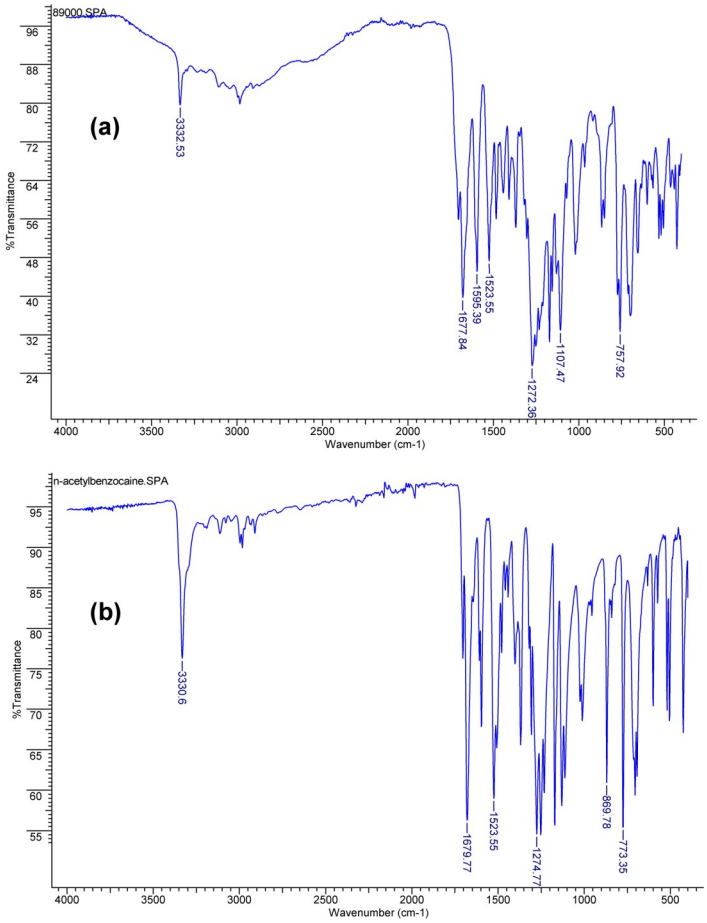

Figure 4 - Infrared spectrum (FTIR-ATR) of (a) illicit exhibit direct and (b) N-acetylbenzocaine standard.

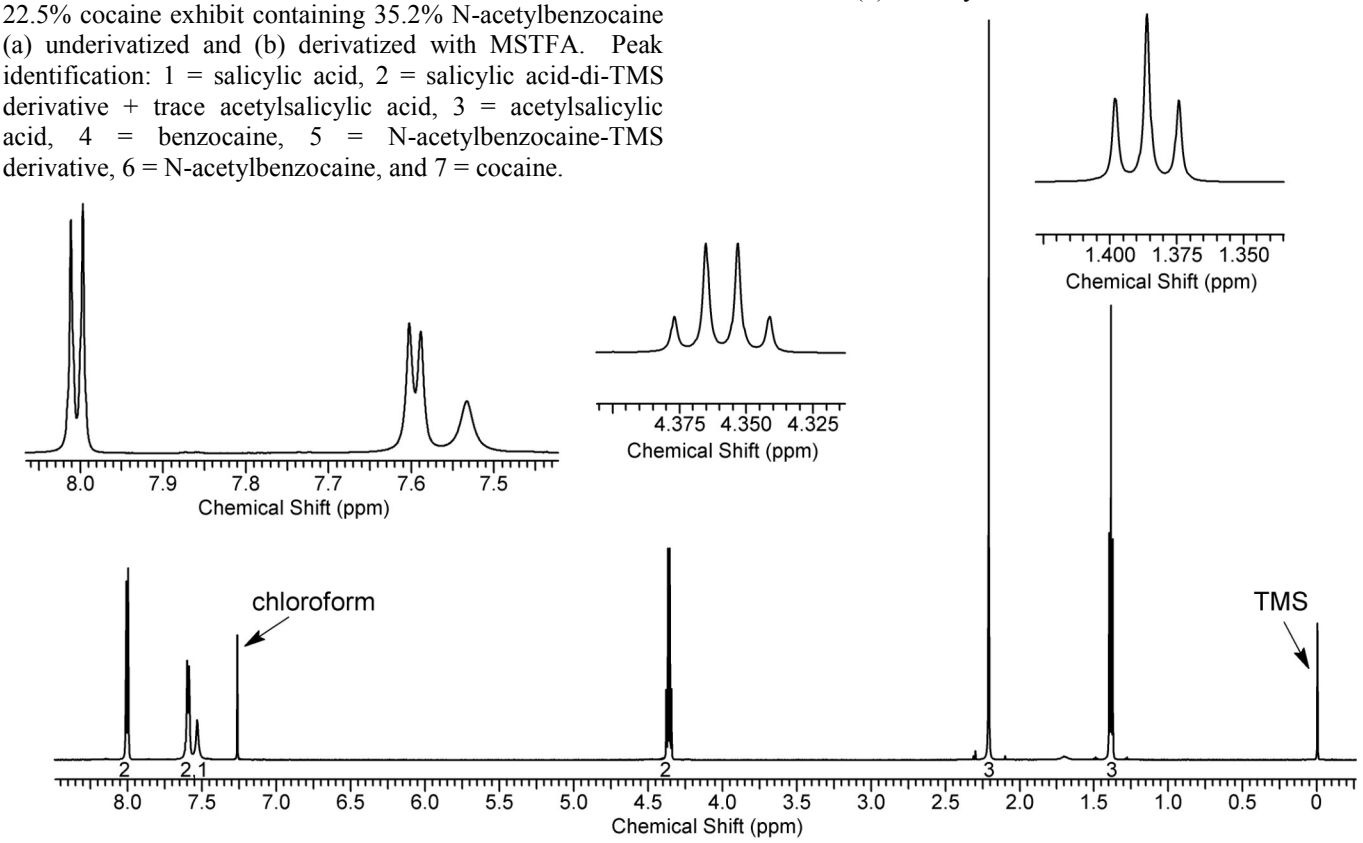

Figure 5 - ^{1}H-NMR spectrum of N-acetylbenzocaine.

Table 1 - ^{1}H-NMR and ^{13}C-NMR chemical shifts (in ppm) and splitting patterns of N-acetylbenzocaine. Samples run in $CDCl_3$ with TMS as the reference compound for 0 ppm.

Number	Benzene Ring	Proton	Carbon
1	C	---	
2	CH	8.00 d (8.5 Hz)	130.8
3	CH	7.60 d (8.5 Hz)	118.7
4	C	---	142.0
5	CH	7.60 d (8.5 Hz)	118.7
6	CH	8.00 d (8.5 Hz)	130.8
	off C-1		
1	O-**CH_2**-CH_3	4.36 q (7.2 Hz)	60.9
2	O-CH_2-**CH_3**	1.38 t (7.2 Hz)	14.4
	Off C-4		
1	NH-**C**(C=O)-CH_3	---	168.5
2	NH-C(C=O)-**CH_3**	2.21 s	24.8

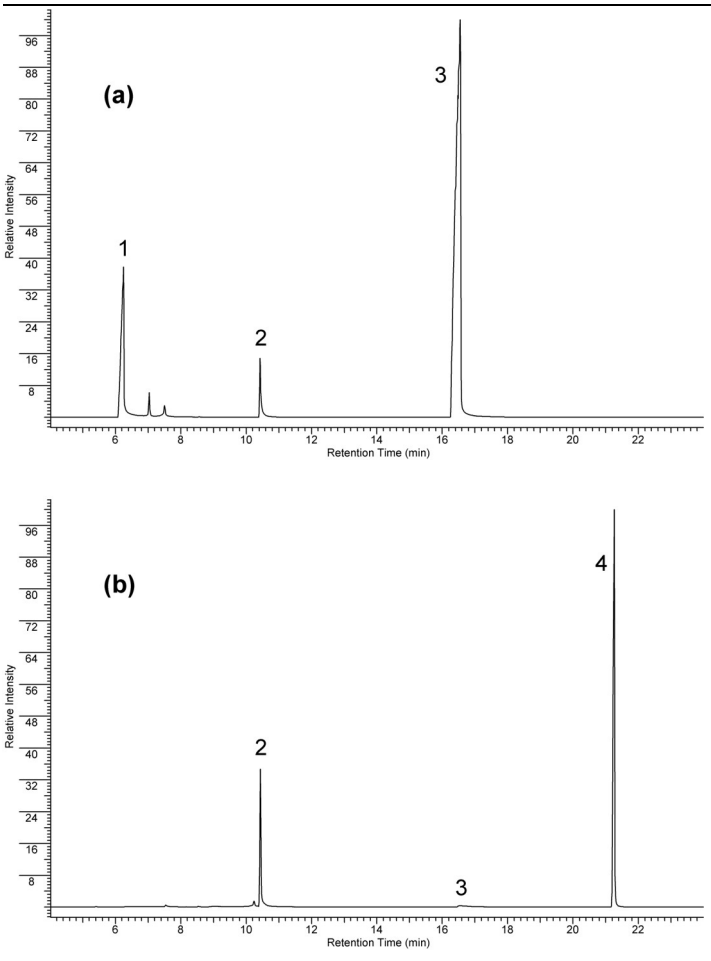

Figure 6 - Partial reconstructed total ion chromatogram of a (a) mixture of benzocaine and acetylsalicylic acid which had been heated overnight neat, and (b) mixture of cocaine HCl, benzocaine, and acetylsalicylic acid in chloroform injected immediately. Peak identification: 1 = salicylic acid, 2 = benzocaine, 3 = N-acetylbenzocaine, and 4 = cocaine.

salicylic acid moiety of aspirin is an excellent leaving group for nucleophilic addition of a primary aromatic amine. This property has been utilized with 3-aminophenol as a pre-column reaction for the determination of aspirin in the presence of acetaminophen by reversed-phase HPLC [10]. In order to confirm that the N-acetylbenzocaine in the illicit exhibit could be produced from transacetylation, a dry mixture of benzocaine and acetylsalicylic acid were warmed overnight (see Experimental section) and then examined via GC/MS (Figure 6a). As illustrated in the reconstructed total ion chromatogram, N-acetylbenzocaine (Peak #3) predominates, with lesser amounts of salicylic acid (Peak #1) and benzocaine (Peak #2). Finally, to rule out the possibility of injection port formation of N-acetylbenzocaine, a mixture of cocaine HCl, benzocaine, and acetylsalicylic acid were dissolved in chloroform and injected. Of note, a trace amount of N-acetylbenzocaine was produced (Figure 6b, Peak #3), indicating that it can be produced as an analytical artifact at low levels under these conditions.

Conclusions

Analytical data is presented to assist other forensic laboratories that encounter N-acetylbenzocaine in case exhibits. N-Acetylbenzocaine can be readily formed from benzocaine and acetylsalicylic acid with heat. Care must be taken in identifying N-acetylbenzocaine, since it can also be formed as an injection port artifact.

Acknowledgements

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