TECHNICAL NOTE

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Formation of an Interfering Substance, 3,4-Dimethyl-5-Phenyl-1,3-Oxazolidine, During a Pseudoephedrine Urinalysis*

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ABSTRACT: During fatal aviation accident investigations, biosamples from the victims are submitted to the FAA Civil Aeromedical Institute (CAMI) for drug analysis. In the process of one such analysis by CAMI, an unknown substance was found in a urine sample. Simultaneous screening by thin layer chromatography (TLC) and gas chromatography/FID (GC/FID) suggested the presence of pseudoephedrine. A subsequent routine confirmation analysis of a separate urine aliquot by GC Fourier transform infrared (GC/FTIR) and GC mass spectrometry (GC/MS) indicated that the retention times of the unknown substance matched with those of pseudoephedrine. However, its infrared and mass spectra were different—the —OH and —NH groups were missing, a C—O—C group was present, and the molar mass was 12 atomic mass units (amu) more than that of pseudoephedrine. A subsequent literature search suggested that ephedrine-like amines react with aldehydes to form oxazolidines. Therefore, the 12-amu increase could be accounted for by condensation of pseudoephedrine with formaldehyde. Since this aldehyde is present in various grades of methanol and ethyl acetate, and these solvents were used during the solid-phase extraction, 3,4-dimethyl-5-phenyl-1,3-oxazolidine was synthesized by using (+)-pseudoephedrine·HCl and formaldehyde. The analytical findings of the synthesized compound were consistent with those of the unknown interfering substance, confirming that it was the oxazolidine. Aldehyde contaminants in solvents or specimens can transform drugs of interest and may result in misidentification of a compound originally present in specimens. Therefore, chemicals used in analyses should be of the highest available purity, and a multi-analytical approach should be adopted to maintain a high degree of quality assurance.

KEYWORDS: forensic science, forensic toxicology, pseudoephedrine, analysis, artifact, oxazolidine

In fatal aircraft accident investigations, postmortem samples collected from the accident victims at autopsy are submitted to the Federal Aviation Administration's (FAA's) Civil Aeromedical Institute (CAMI) for toxicological evaluation (1). The submitted samples are analyzed for the presence of drugs, including sympathomimetic amines (SMAs), such as ephedrine, pseudoephedrine, and phenylpropanolamine. An unknown substance, which interfered with the analysis of pseudoephedrine, was found during the analysis of a urine sample from a pilot who died in an aviation accident. This compound had the same gas chromatographic retention time as that of pseudoephedrine, but its infrared (IR) and mass spectra (MS) were different. Pseudoephedrine's characteristic O—H and N—H bond stretchings were absent in the IR of the unknown compound and its molar mass was 12 atomic mass units (amu) more than pseudoephedrine.

It is known that ephedrine-like amines undergo cyclization with aliphatic, as well as aromatic, aldehydes to form oxazolidines (2–5). This cyclization reaction involves the condensation of the amines with an aldehyde, with a resulting loss of one water molecule (Fig. 1). The molar mass of the resulting oxazolidine is the total of the masses of the amine plus the aldehyde, minus the mass of water. Therefore, a possible explanation of these analytical findings is the condensation of pseudoephedrine with formaldehyde.

The problem of aldehydic impurities in solvents is not new—for example, the presence of trace amounts of formaldehyde, acetaldehyde, and/or propionaldehyde has been reported in diethyl ether and ethyl acetate (2,6). Formaldehyde is also present in some common grades of methanol. During the analysis of ephedrine, while using diethyl ether as an extraction solvent, as many as three peaks corresponding to oxazolidines of these three aldehydes were observed by Beckett (2). Similarly, acetaldehyde as a contaminant in ethyl acetate has been reported to potentially react with ephedrine, pseudoephedrine, and phenylpropanolamine, thereby potentially affecting their analyses (6).

In view of the initial analytical data and the potential aldehydic contamination of solvents, an oxazolidine was synthesized from pseudoephedrine and formaldehyde. This paper describes the identification, synthesis, and the analytical and chemical properties of 3,4-dimethyl-5-phenyl-1,3-oxazolidine and exemplifies the dangers of aldehyde contaminants in the analysis of ephedrine-like compounds.

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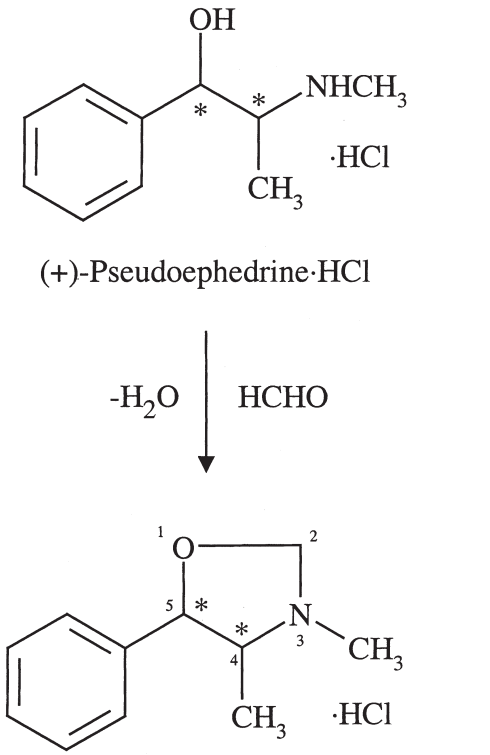

FIG. 1—*Chemical scheme for the synthesis of the oxazolidine using (+)-pseudoephedrine HCl and formaldehyde (HCHO). The asterisks indicate chiral centers (asymmetric carbons) in the molecules.*

Materials and Methods

Materials

All reagents and solvents were of analytical grade and were of the highest available purity. These chemicals, standards, and other agents were obtained from commercial sources. (+)-Pseudoephedrine as hydrochloric acid salt was supplied by Sigma Chemical Co., St. Louis, MO. Supplies for the thin layer chromatography were provided by TOXI-LAB, Inc., Irvine, CA. CS ChemOffice software for the calculation of theoretical NMR spectra was supplied by CambridgeSoft, Cambridge, MA. The ^{1}H and ^{13}C nuclear magnetic resonance spectra were obtained at the University of Southern Mississippi, Hattiesburg, MS.

Oxazolidine—(+)-3,4-Dimethyl-5-phenyl-1,3-oxazolidine·HCl was synthesized (Fig. 1) in our laboratory and characterized by its melting point, elemental analyses (Galbraith Laboratories, Inc., Knoxville, TN), and spectral analyses. To a 50 mL portion of methanol, 2.0 g (9.9 mmol) of (+)-pseudoephedrine·HCl and 1.2 g (40 mmol) of formaldehyde (3 mL of 37%) solution were added. This homogeneous mixture was then stirred for 15 min at the ambient temperature. After stirring, the volume of the mixture was reduced to 2 to 3 mL, using a 40°C water bath and a stream of nitrogen gas. To this concentrate, 2 mL of water was added, and the solution was extracted three times with 8 mL aliquots of chloroform. After drying over sodium sulfate, the chloroform extract was transferred and then evaporated to dryness. The white solid was recrystallized from a diethyl ether-methanol solvent system. The product was filtered and dried under vacuum at 40°C for 10 min, yielding a white crystalline powder (1.7 g; 82%) melting at 173 to 174°C (dec.).

The calculated elemental analysis for (+)-3,4-dimethyl-5-phenyl-1,3-oxazolidine, $C_{11}H_{16}ClNO$, is: C, 61.82%; H, 7.55%; N, 6.55% and the observed elemental analysis is: C, 61.44%; H, 7.61%; N, 6.55%. The MS (70 eV) m/z (relative intensity): 71 (100%); 56 (26%); 91 (10%); 117 (7%).

Screening

Thin Layer Chromatography—The urine sample was screened for the presence of drugs following the TOXI-LAB's standard recommended procedure for the analysis of basic drugs.

Gas Chromatography—A 5 mL portion of urine was mixed with 500 ng of the internal standard propylamphetamine and was then subjected to liquid-liquid basic extraction with ammonium hydroxide (>10.00 pH) followed by extraction with 5 mL of chloroform. The chloroform layer was transferred to another test tube and was washed with 5 mL of 0.1 N hydrochloric acid. The acidic aqueous layer was transferred and made basic (>10.00 pH) with ammonium hydroxide. To this mixture, an equal volume of chloroform was added and gently mixed. The chloroform layer was removed and evaporated down to approximately 25 μL using a stream of nitrogen. One microliter of the evaporate was injected into a 5890 Model Series II Hewlett Packard gas chromatograph (GC), functioning under our standard laboratory GC screening conditions. The GC was equipped with flame ionization and nitrogen phosphorus detectors (FID/NPD). A crosslinked 5% phenyl methyl silicone column (15 m × 0.25-mm i.d.; 0.25 μm film thickness) was used.

Confirmatory Analysis

A separate aliquot of urine used to confirm the initial screening tests was subjected to solid-phase extraction, following the manufacturer's (Bond Elut Certify™, Varian Sample Preparation Products, Harbor City, CA) recommended procedure for isolation of basic drugs. During this procedure, methanol was used in various steps. The eluates were evaporated and subjected to analyses using a Hewlett Packard 5890 Series II gas chromatograph equipped with a HP 5965B infrared detector (GC/FTIR) and using a Hewlett Packard 5890 Series II gas chromatograph in combination with a 5989A mass spectrometer (GC/MS). Further analysis was performed on the extract using the GC/MS PCI technique.

The column used for the GC/FTIR analysis was a Hewlett Packard HP1 crosslinked 100% methyl siloxane column (15 m × 0.32 mm i.d.; 1 μm film thickness). For the GC/MS analysis, a Hewlett Packard ULTRA 1 crosslinked 100% methyl siloxane column (12 m × 0.2 mm i.d.; 0.33 μm film thickness) was used. For both analyses, the injection volume was 1 μL in the splitless mode, with a purge time of 0.5 min. The GC oven temperature was increased from 70°C to 160°C at 15°C/min and then to 290°C at 40°C/min. The final temperature of 290°C was maintained for 1.75 min, totaling an 11-min run time. Helium was the carrier gas with a flow of 1 mL/min. The injector temperature was maintained at 250°C. The transfer line was set at 280°C.

Results and Discussion

Initial screening of the urine specimen by TOXI-LAB revealed a spot consistent with the characteristic Rf value (≈0.14) and the

color of pseudoephedrine/ephedrine. Based on the retention time, the GC/FID/NPD analysis of the liquid-liquid extract further suggested that pseudoephedrine was present in the specimen. The retention time for the analyte of interest from the solid-phase extract matched that of pseudoephedrine during the GC/FTIR and GC/MS confirmation analyses. Gas chromatography-Fourier transform infrared spectrometry (GC/FTIR) revealed the presence of characteristic absorptions of aromatic C—H, monosubstituted benzene, —CH_2—, and —CH_3 groups. The characteristic pseudoephedrine O—H stretch at 3600 cm^{-1} and the N—H stretch at 3400 cm^{-1}, however, were absent in the analyte spectrum (Figs. 2*a* and 2*b*), and a strong absorbance associated with an ether (C—O—C) stretch at 1100 cm^{-1} was observed (Fig. 2*a*). The gas chromatography mass spectrometry (GC/MS) electron ionization (EI) spectra disclosed a base peak of 71 amu (Fig. 3*a*), whereas pseudoephedrine has a base peak at 58 amu (Fig. 3*b*). Positive chemical ionization (PCI) mass spectrometry of the unknown substance exhibited a mass of 178 amu (M + 1) (Fig. 4), indicating its actual molar mass to be 177 amu, which is 12 amu more than the

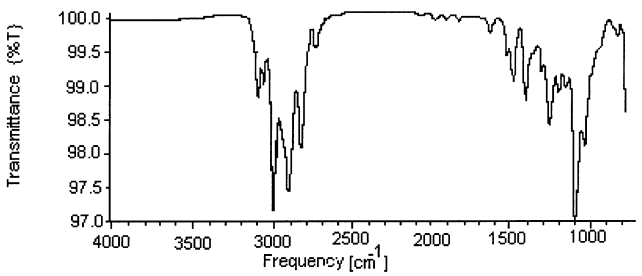

FIG. 2*a*—*Infrared spectrum obtained in the process of the urine extract GC/FTIR analysis.*

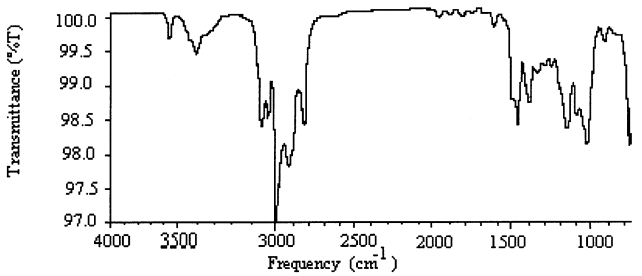

FIG. 2*b*—*Infrared spectrum of (+)-pseudoephedrine subjected to the GC/FTIR analysis.*

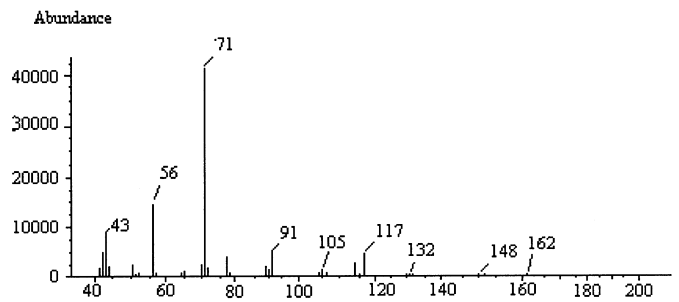

FIG. 3*a*—*Mass spectrum obtained after the GC/MS analysis of the urine extract.*

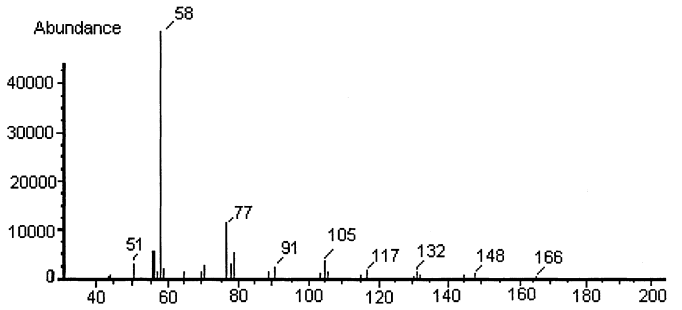

FIG. 3*b*—*Mass spectrum of (+)-pseudoephedrine subjected to the GC/MS analysis.*

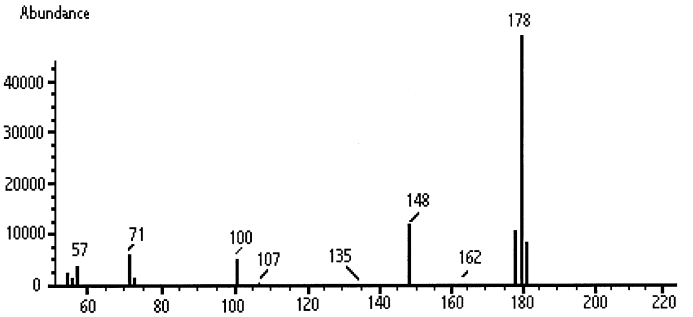

FIG. 4—*PCI-based GC mass spectrum obtained for the urine extract.*

Carbon 13 NMR in DMSO

Atom	Observed (PPM)	Calculated (PPM)
C(3)	10.6	12.6
C(1)	35.6	32.8
C(2)	66.7	68.4
C(5)	84.0	85.6
C(4)	85.3	91.7
C(9)	127.1	125.8
C(7)	128.5	128.3
C(8)	129.2	128.6
C(6)	135.9	138.8

NMR in DMSO

Atom	Observed (PPM)	Calculated (PPM)
CH_3(3)	1.00	1.10
CH_3(1)	2.12	2.27
CH(2)	3.10	3.50
CH(5)	4.45	4.35
CH(4)	4.48	4.44
CH(9)	7.04	7.19
CH(7)	7.06	7.19
CH(8)	7.11	7.19

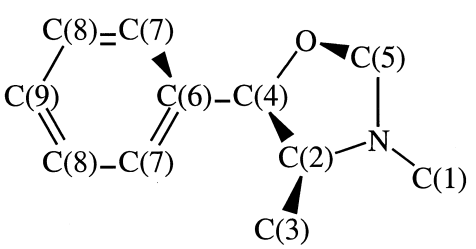

FIG. 5—*Comparison of observed NMR spectra with calculated NMR spectra.*

molar mass of pseudoephedrine (165 amu). The synthesized oxazolidine showed IR and MS spectra matching that of the unknown, interfering substance. The experimentally determined ^{1}H and ^{13}C nuclear magnetic resonance spectra of the synthesized compound were consistent with the theoretically calculated NMR values corresponding to the oxazolidine molecule (Fig. 5). Additionally, elemental analysis of the synthesized oxazolidine agreed with the expected values.

Findings from this study revealed difficulties associated with formaldehyde contamination in the chemicals used for analysis. The presence of formaldehyde in the analysis of pseudoephedrine could result in the conversion of pseudoephedrine to another compound. Identified as 3,4-dimethyl-5-phenyl-1,3-oxazolidine (Fig. 1), the interfering substance is formed by a reaction between formaldehyde and pseudoephedrine, involving the —OH and —NH— groups of pseudoephedrine. This type of reversible reaction has been reported with ephedrine-like amines and aldehydes, forming oxazolidines (2–6). The reaction is a condensation between an aldehyde and a secondary amine leading to the formation of an intermediate iminium ion, which subsequently reacts with an active hydrogen atom on the hydroxyl group leading to the formation of the five-membered cyclic oxazolidine with a 12-amu increase (Fig. 1). In this reaction, the chirality of the pseudoephedrine's two asymmetric carbons does not change, suggesting that the stereochemistry of the formed compound remains the same as that of the starting material. In those SMAs—for example, amphetamine and methamphetamine—wherein the —OH group is absent, the formation of the oxazolidines is not possible.

Although the source of formaldehyde contamination could not be established, the findings from this study clearly emphasize the importance of using high-quality reagents and of the identification of unknowns by multi-analytical approaches, including spectral analyses. The presence of contaminants in extraction solvents, or in any other chemical, can result in a false negative or false positive analytical finding. If the substance formed from the contaminant is a known drug, then the findings could be misleading. Therefore, it is prudent that chemicals of known purity be used during drug analysis to maintain a high degree of quality assurance for a laboratory.

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