Technical Note

Identification of Levamisole Impurities Found in Illicit Cocaine Exhibits

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ABSTRACT: 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole and 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one, known levamisole degradation products, were identified in a "crack" cocaine exhibit. Spectroscopic and chromatographic data are provided for both compounds, and their presence in the sample is discussed.

KEYWORDS: Levamisole, Degradation, Cocaine Base, "Crack" Cocaine, Impurities, 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole, 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one, Forensic Chemistry

Introduction

This laboratory recently received a 1 gram portion of a "crack" cocaine (cocaine base) exhibit from another laboratory for the purpose of identifying an unknown component. The exhibit contained 79% cocaine base, 6% levamisole, and 3% of an unknown compound. The unknown had an apparent molecular weight of 202 Daltons based on the mass spectrum generated by the original laboratory, and was suspected to be a levamisole-related impurity. Upon screening, the exhibit was found to also contain trace amounts of a second unknown compound, suspected to be another levamisole-related impurity. Levamisole (Figure 1), an antineoplactic (cancer chemotherapy drug), has been a cocaine adulterant for nearly 5 years [1], but this is the first report of suspected levamisole impurities in illicit cocaine. The prevalence of levamisole in cocaine hydrochloride bricks has increased dramatically over the past year, and is currently found in 30% of all seizures (Figure 2). Herein, we report the preparative isolation, gas and liquid chromatographic-mass spectrometry, and nuclear magnetic resonance spectroscopy of both impurities. The major unknown compound (6-phenyl-2,3-dihydroimidazo[2,1b]thiazole) was confirmed via comparison to an authentic standard, while the trace unknown compound (3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one) was synthesized to verify its identity.

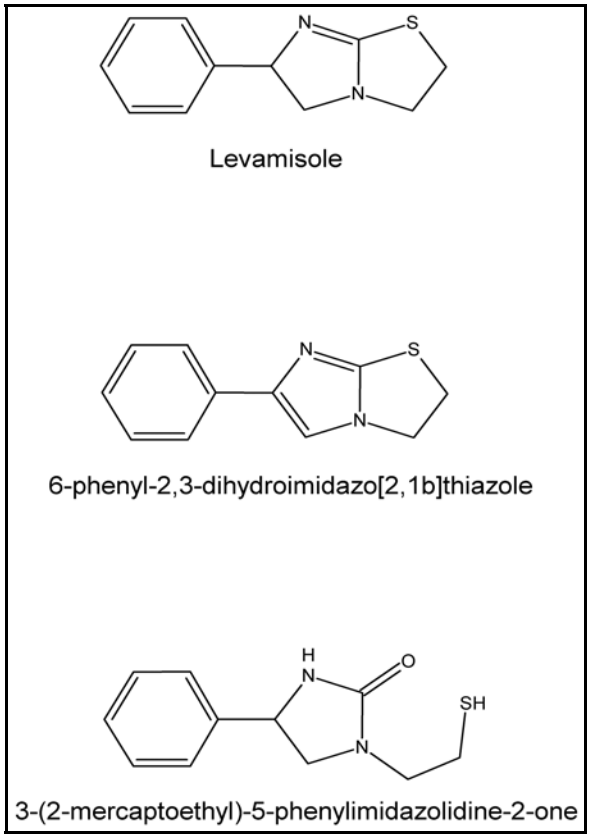

Figure 1.

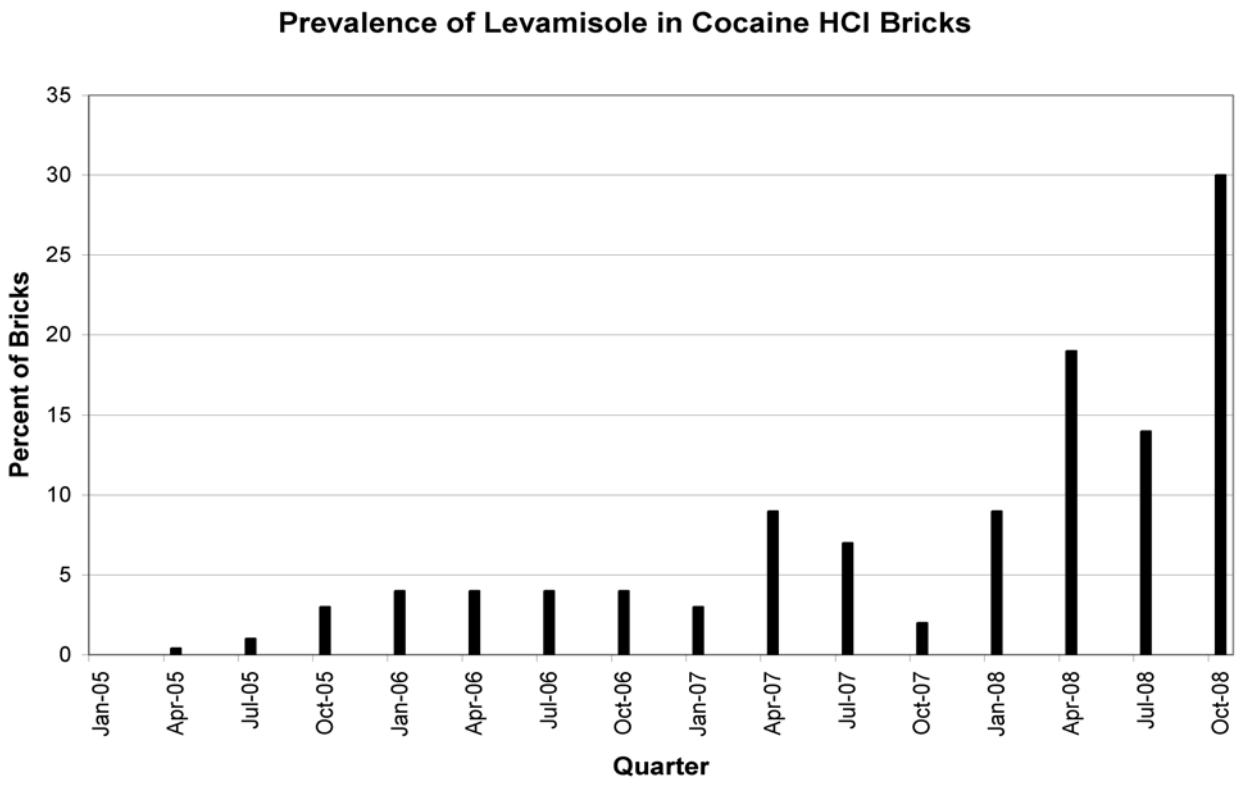

Figure 2. Prevalence of Levamisole in Cocaine Hydrochloride Bricks over the past 4 Years.

Experimental

Solvents, Chemicals, and Materials: All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of reagent-grade quality and were products of Sigma-Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4% (w/w). Levamisole was part of the authentic reference collection of the DEA Special Testing and Research Laboratory. A reference standard of 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole was obtained from LGC Standards (Luckenwalde, Germany).

Gas Chromatography/Mass Spectrometry (GC/MS): GC/MS analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC system 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^{O}C (no initial hold); program rate, 6^{O}C/min; final temperature, 300^{O}C; final hold, 5.67 min. The injector was operated in the split mode (21.5 : 1) and at a temperature of 280^{O}C. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and a scan rate of 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280^{O}C and 230^{O}C, respectively.

Liquid Chromatography/Mass Spectrometry (LC/MS): Molecular weight information derived from $[M+H]^+$ was obtained using a Waters (Milford, MA) 2525 HPLC pump fitted with a XTerra MS 150 mm x 4.8 mm, 5 µm, C-18 column. The sample was diluted to a concentration of approximately 0.2 mg/mL, and the injection volume

was 0.5 mL per run. The flow was optimized at 1.0 mL/min, using the following reversed-phase gradient solvents: (A) Water containing 0.1% trifluoroacetic acid, and (B) Acetonitrile. The linear gradient started at 95% A and 5% B; changed to 75% A and 25% B over 20 min, held 15 min; then changed to 5% A and 95% B over 25 min; and finally returned to 95% A and 5% B for 1 min. The HPLC eluent was introduced into a Waters Micromass ZQ single quadrupole mass spectrometer using Electrospray Ionization (ESI) with positive ion detection. The detector operated in the scan range of 40 - 500 mass units, a scan time of 0.5 sec, and an inter-scan delay of 0.1 sec.

Preparative Isolation of 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole via Alumina Column Chromatography: Approximately 900 mg of the illicit cocaine sample containing about 3% (~27 mg) of target compound was dissolved in a minimal amount of $CHCl_3$ and eluted on a glass chromatographic column (25 cm x 1.0 cm i.d.) containing 15 g of basic alumina (150 mesh). The column was eluted with 20 mL each of the following solvent combinations: 1) $CHCl_3$, 2) $CHCl_3$/acetone (85 : 15), 3) $CHCl_3$/acetone (1 : 1), and acetone. Ten mL fractions were collected and examined via GC/MS. Fractions 2 and 3 contained 17 - 22% pure target compound and were combined and evaporated to dryness. The residue was dissolved into a minimal amount of $CHCl_3$/hexane (1 : 1) and was chromatographed again on 15 g of basic alumina (150 mesh). The column was eluted with 20 mL each of the following solvent combinations: 1) $CHCl_3$/hexane (1 : 1), 2) $CHCl_3$, 3) $CHCl_3$/acetone (40 : 1), 4) $CHCl_3$/acetone (20 : 1), 5) $CHCl_3$/acetone (15 : 1), 6) $CHCl_3$/acetone (10 : 1), and 7) $CHCl_3$/acetone (6 : 1). Ten mL fractions were collected and examined via GC/MS. Fraction 5 contained the majority of the target compound (but also contained some levamisole and cocaine), and was evaporated to dryness. The residue was washed with 2 - 3 mL of petroleum ether (20 - 40^{O}C boiling range) to remove the cocaine, and was then dried to provide a white powder (20 mg, 67% 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole and 33% levamisole).

Nuclear Magnetic Resonance Spectroscopy (NMR): Proton (^{1}H) NMR spectra were obtained on a Varian (Palo Alto, CA) Inova 600 MHz NMR using a 5 mm Varian Nalorac Z-Spec broadband, variable temperature, pulse field gradient (PFG) probe. The compounds were dissolved in deuterochloroform ($CDCl_3$) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference. The temperature of the samples was maintained at 25^{O}C. Standard Varian pulse sequences were used to acquire the spectra. Data processing was performed using Applied Chemistry Development software (ACD/Labs, Toronto, Canada).

Synthesis of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one: Levamisole hydrochloride (25 mg, 0.104 mmol) was dissolved in water (5 mL), adjusted to pH 8 with aqueous $NaHCO_3$ (1 mL), and microwaved at 1200 watts until all the water had boiled off (about 2 - 3 minutes). The residue was dissolved in $CHCl_3$ (10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo* to give the title compound as a white powder (22 mg, 95%). The reaction was repeated at pH 10 using aqueous Na_2CO_3, and gave identical results.

Results and Discussion

GC/MSD analysis of the exhibit was first conducted as a cursory assessment. Examination of the reconstructed total ion chromatogram (Figure 3a, Table 1) indicated a compound (Peak #4) closely related to levamisole was present. Peak #4 represented approximately 3% of the total ion current. Its mass spectrum (Figure 4a) produced an apparent molecular ion at *m/z* 202. The spectrum was markedly similar to levamisole (Figure 4b), with fragment ion shifts of minus one to two mass units for several ions, thus suggesting a levamisole-like compound with incorporation of another double bond. It did not form a TMS derivative, indicating no labile protons within the molecule. The molecular weight for this compound was confirmed via LC/MS, yielding a $[M+H]^+$ at *m/z* 203, consistent with the molecular weight assignment of 202. This compound was semi-isolated as described in the Experimental section and examined via ^{1}H-NMR. The chemical shifts obtained were consistent with the loss of two protons within the imidizole ring and suggested that the compound was 6-phenyl-2,3-dihydro-imidazo[2,1b]thiazole. A reference standard of this compound was obtained, and its retention time and mass spectrum were identical to the unknown (Figure 1).

A trace component (Figure 3a, Peak #3) was also noted in the GC/MSD analysis, having an apparent molecular ion at *m/z* 222 (Figure 5a). Upon derivatization with MSTFA, this compound formed both a mono-TMS and di-TMS derivative (Figure 3b, Peaks #2 & #6), with molecular ions at *m/z* 294 (Figure 5b) and *m/z* 366 (Figure 5c), respectively. These results indicated that two labile protons were present. A mass difference of +18 Daltons from levamisole, coupled with two labile protons, suggested that the compound was an oxidation by-product of levamisole. Since 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one had been previously reported as an oxidative by-product of levamisole in aqueous solutions [2-4], it was synthesized as described in the Experimental section. The retention times and mass spectra of the synthesized standard (both underivatized and derivatized) were identical to the unknown (Figure 1).

Some Colombian-run cocaine hydrochloride laboratories have been adding levamisole to cocaine hydrochloride for nearly 5 years. Over that time period, the levamisole has always appeared to be of pharmaceutical-grade quality. Significant amounts of process impurities are rarely encountered in pharmaceutical drug products. This exhibit contained 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole at a concentration of about 50% relative to levamisole, which is quite remarkable. This impurity (and the trace amount of 3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one) may have arisen from two possible sources: 1) A poorly processed or waste batch of pharmaceutical levamisole; and/or 2) Degradation of levamisole during the conversion of cocaine hydrochloride into "crack" cocaine.

The formation of significant amounts of 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole from levamisole during the conversion of cocaine hydrochloride into "crack" cocaine as currently practiced seems unlikely. The stability of levamisole in aqueous solutions has been studied at length [2-4]. In those works, the formation of up to four degradation products were tracked as a function of pH and temperature. 6-Phenyl-2,3-dihydroimidazo[2,1b]-thiazole was not detected even when levamisole was boiled to dryness in basic aqueous solutions (i.e., at pH 7 to 10). However, 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole is also a synthetic by-product from the pharmaceutical production process, due to an undesired cyclization of the first intermediate product [5]. Due to the very high relative concentration of this compound to levamisole in this sample, it appears that an impure or waste batch of levamisole made its way into the illicit drug trade.

However, as was demonstrated in the Experimental section, 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one is nearly quantitatively created from levamisole when boiled to dryness in basic aqueous solutions (i.e., at either pH 8 or 10). Therefore, the trace amount of 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one in the sample may be attributed to its formation as a by-product from levamisole during the conversion of cocaine hydrochloride into "crack" cocaine. In this case, the water was not boiled off during the "crack process," or all of the levamisole would have been converted to this compound. The presence of large amounts of 3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one in "crack" cocaine exhibits would be evidence of boiling the "crack" cocaine conversion solution to dryness.

The physiological effects and consequences of smoking "crack" cocaine adulterated with levamisole and contaminated with 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole and 3-(2-mercaptoethyl)-5-phenylimidazol-idine-2-one are unknown.

References

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Table 1. Retention Times (RT) and Relative Retention Times (RRT) of Levamisole and Related Impurities [a.]

Compound	RT (min)	RRT (min)
Levamisole	17.25	0.81
222-mono-TMS*	19.09	0.90
222*	19.77	0.93
202**	20.99	0.98
Cocaine	**21.30**	**1.00**
222-di-TMS*	22.39	1.05

[a] Conditions Detailed in the Experimental Section.
* 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one
** 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole

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[Figures 3 - 5 Follow.]

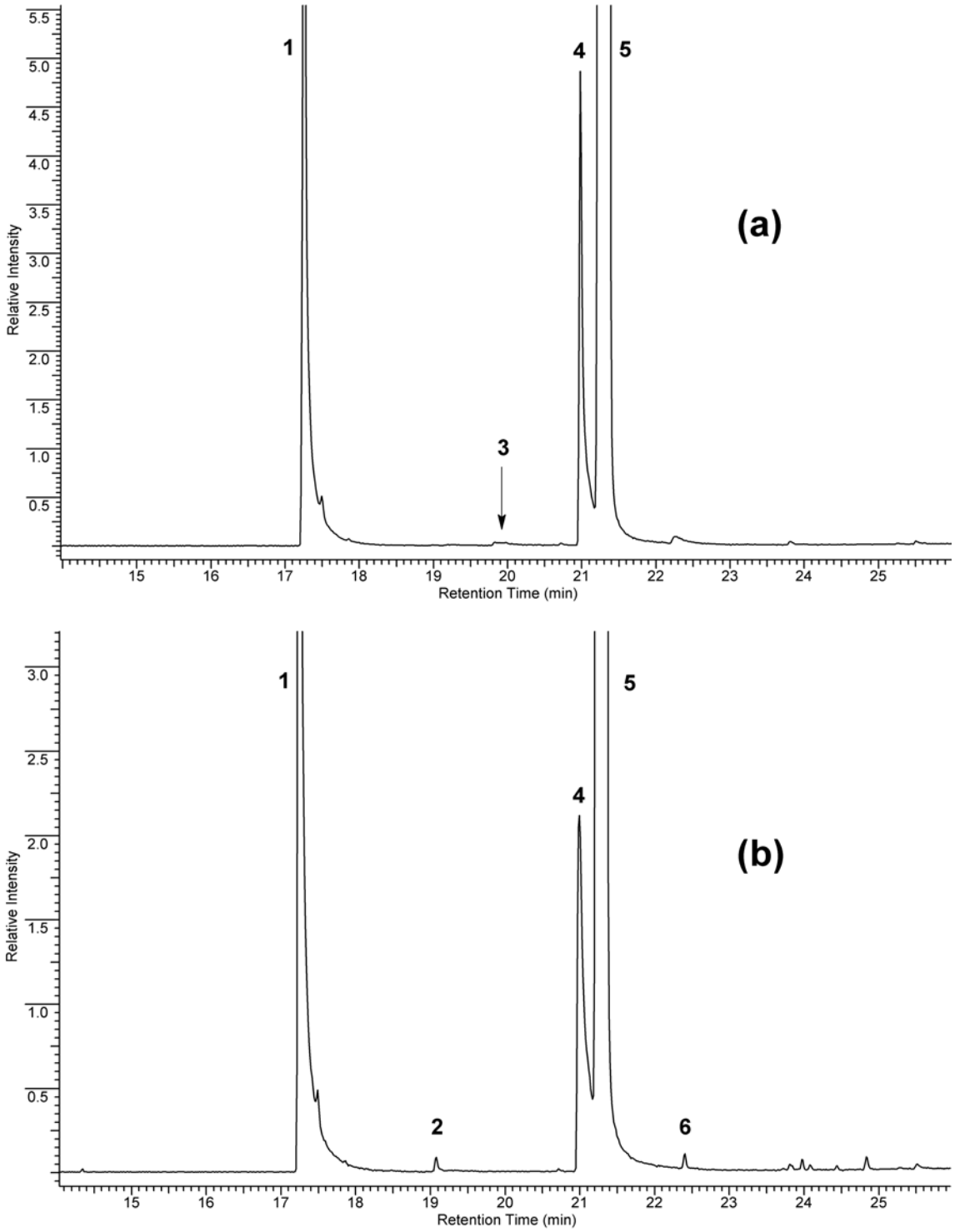

Figure 3. Partial Reconstructed Total Ion Chromatograms of a Cocaine Base Exhibit Containing Levamisole Impurities. Upper (A) Is Underivatized and Lower (B) Is Derivatized. Peak Identification: 1 = Levamisole, 2 = TMS Derivative of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one, 3 = 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one, 4 = 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole, 5 = Cocaine, 6 = di-TMS Derivative of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one.

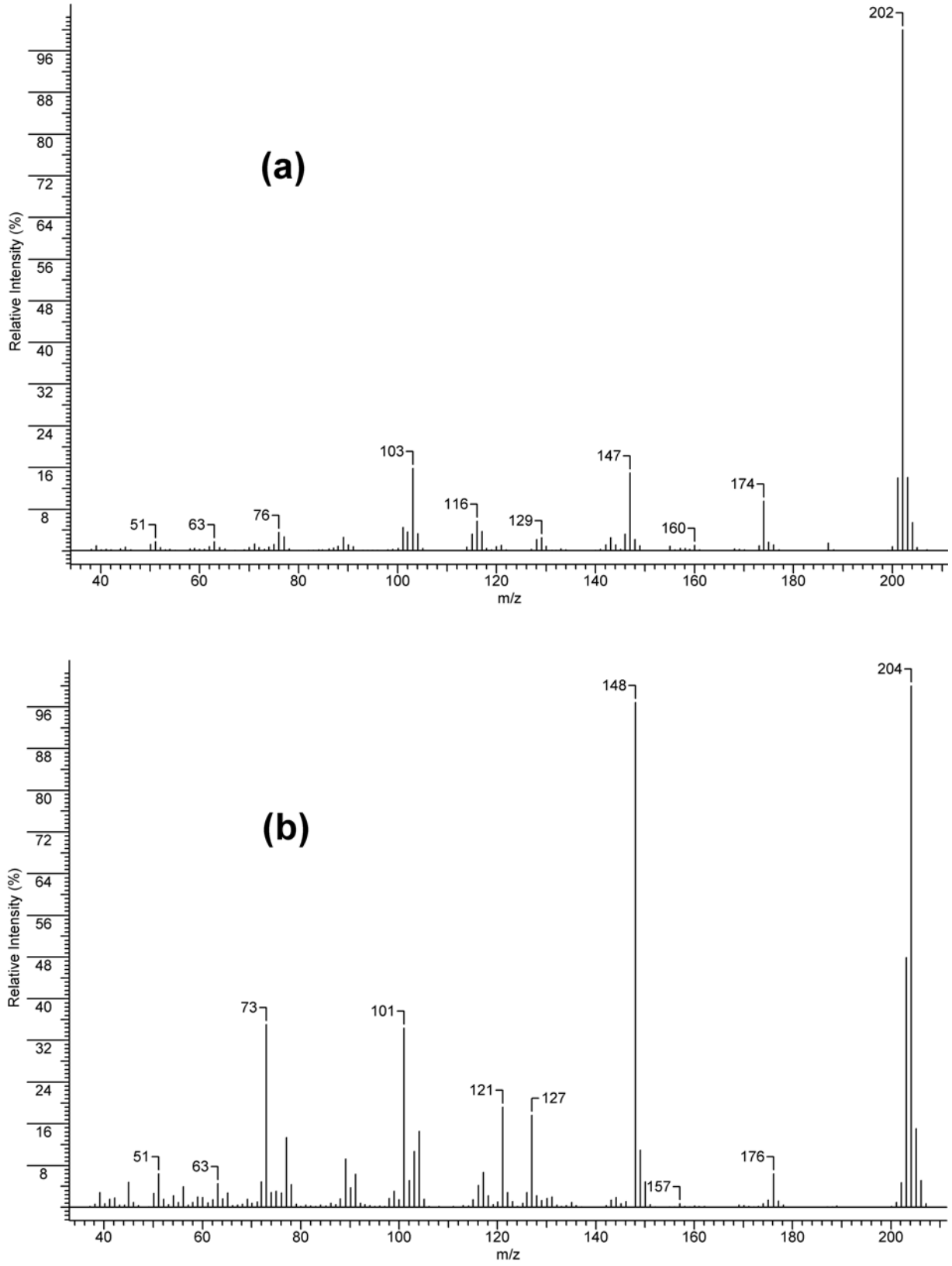

Figure 4. Electron Ionization Mass Spectrum of (A) 6-Phenyl-2,3-dihydro-imidazo[2,1b]thiazole; and (B) Levamisole.

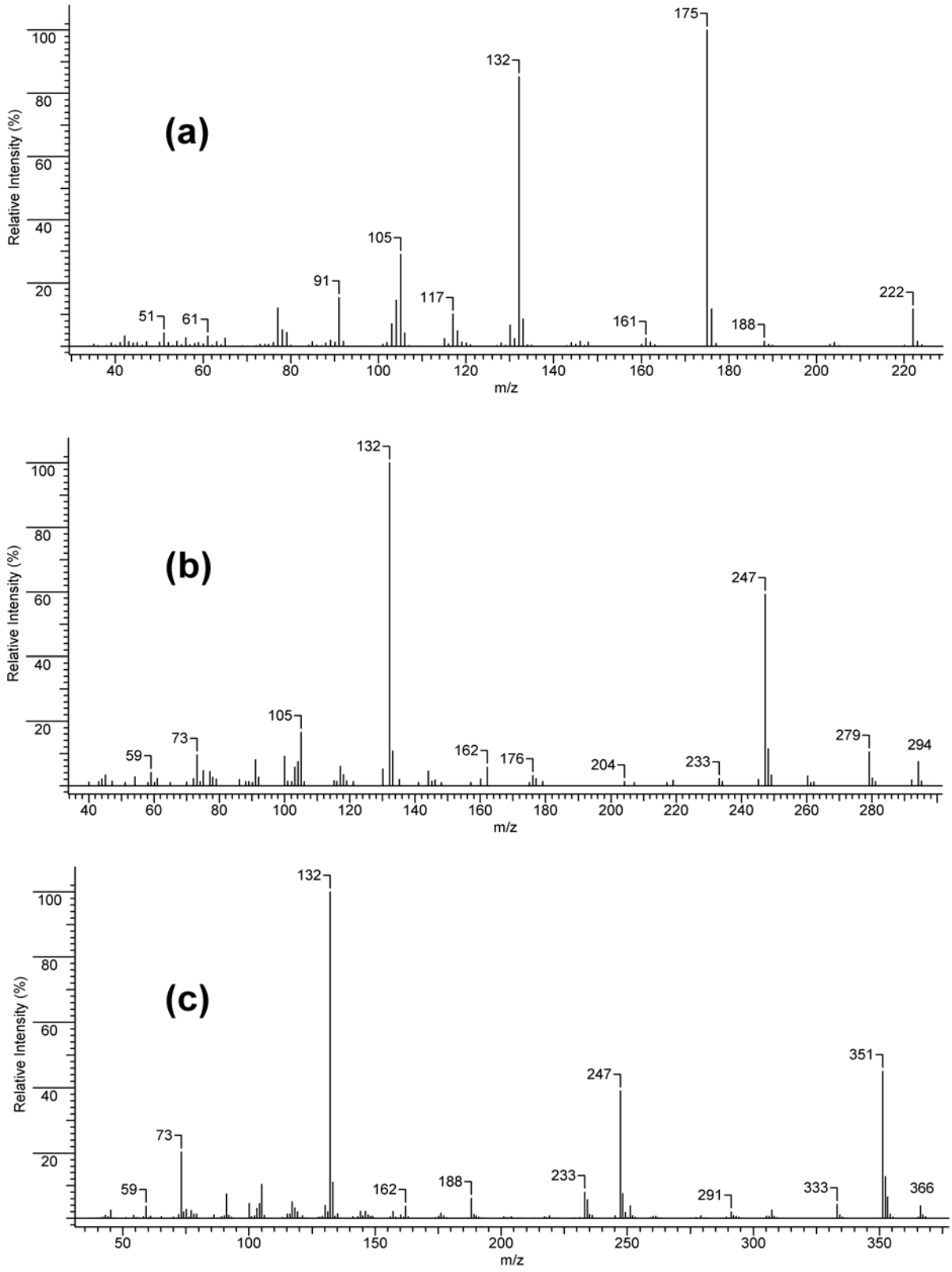

Figure 5. Electron Ionization Mass Spectrum of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one: (A) Underivatized, (B) mono-TMS Derivative, and (C) di-TMS Derivative.