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Neutral Heroin Impurities from Tetrahydrobenzylisoquinoline Alkaloids

ABSTRACT: Laudanosine, reticuline, codamine, and laudanine are members of the tetrahydrobenzylisoquinoline family of natural products. These alkaloids are present in the opium poppy, *Papaver somniferum*, and are subsequently found as impurities in clandestinely processed morphine. Morphine is then synthesized to heroin using hot acetic anhydride. During the course of this study, it was determined that these four tetrahydrobenzylisoquinolines undergo degradation to a series of 18 neutral impurities when subjected to hot acetic anhydride. Based on the degradation pathway, these new impurities were categorized into two sets of impurities called the C1-acetates compounds and the stilbene compounds. Synthesis, isolation, and structural elucidation information is provided for the tetrahydrobenzylisoquinoline alkaloids, and the new neutral impurities have been studied. Several hundred authentic heroin samples were analyzed using an established heroin signature program method. This methodology features the detection of trace neutral impurities present in heroin samples. It was determined that all 18 new impurities were detected in various quantities in four different types of heroin samples. Analytical results featuring these new impurities are reported for South American-, Southwest Asian-, Mexican-, and Southeast Asian-type heroin samples. These new impurities, coupled with other established forensic markers, enhance the ability to classify illicit heroin samples.

KEYWORDS: forensic science, laudanosine, reticuline, codamine, laudanine, tetrahydrobenzylisoquinoline, synthesis, isolation, chemical analysis, impurities, rotamers, heroin

Trace impurity analysis of heroin continues to be an important method for gathering intelligence data on seized heroin samples. This method has been used by forensic laboratories throughout the world to profile illicit heroin samples (1). This chemical analysis, as performed by the United States Drug Enforcement Administration and known as the Heroin Signature Program, has been separated into three distinct analyses based on the intrinsic chemistry of the sample. The first signature features chemical ratio analysis of the basic alkaloid impurities present in heroin, such as acetylcodiene, O6-monoacetylmorphine, O3-monoacetylmorphine, noscapine, and papaverine. This analysis has traditionally been performed with methods developed using high-performance liquid chromatography (HPLC) (2) and most recently by capillary electrophoresis (CE) (3). The second signature involves the selective extraction and gas chromatography–mass spectrometry (GC/MS) semiquantitation and analysis of the trace-level acidic and neutral impurities present in heroin. This analysis was originally performed using GC/FID technology (4,5). The third analysis is GC/MS-headspace analysis of occluded solvents trapped in the crystal matrix of the heroin material (6). This analysis tracks the organic solvents used to produce the final heroin product.

Trace neutral impurities found in heroin are generally a result of a chemical transformation or degradation of morphine and other trace alkaloids formed in the hot acetic anhydride reaction. Well-known examples of this transformation or degradation include: the formation of triacetylnormorphine from normorphine or morphine N-oxide (7,8), the formation of diacetylnorcodeine from norcodiene or codeine N-oxide (7), the formation of substituted phenanthrenes from the facile and complete degradation of thebaine (9), and the formation of late-eluting *N*-acetylnornarceine-like impurities from the degradation of noscapine (10).

The benzylisoquinolines are known to be key biosynthetic precursors to many of the naturally occurring alkaloids, including morphine and codeine (11). The benzylisoquinolines feature a fused A/B ring system along with a benzylic C ring. These compounds are either completely aromatic like papaverine **1** or contain a reduced 1, 2, 3, 4 tetrahydro-B ring like laudanosine **2**, reticuline **3**, codamine **4**, and laudanine **5**, also known as racemic laudanidine. These compounds are illustrated in Fig. 1. Compounds **2–5** are depicted as racemic compounds because of reports that they exist in opium as a mixture of optical isomers (12–14).

Because of their inherent amphoteric nature and chemical similarity to morphine, compounds **2–5** are carried over as trace impurities in the isolation of morphine from opium. These compounds undergo a facile degradation process to neutral impurities that are present in heroin samples. Herein, we report the preparation and purification of the tetrahydrobenzylisoquinoline compounds, the identification of their corresponding neutral impurities, and the forensic trends of these neutral compounds from the GC/MS analysis of authentic heroin samples.

Experimental

Materials and Methods

Laudanosine was synthesized from tetrahydropapaverine base. Tetrahydropapaverine base was made from its corresponding hydrochloride salt obtained from Sigma Chemical Co. (St. Louis, MO) (Lot 120H038). Codamine and laudanine were synthesized from laudanosine base. Reticuline was obtained from Tasmanian Alkaloids Inc. (Westbury, Tasmania) (Lot 182). All other reagents and chemicals were of reagent-grade quality, or better, and were used without further purification.

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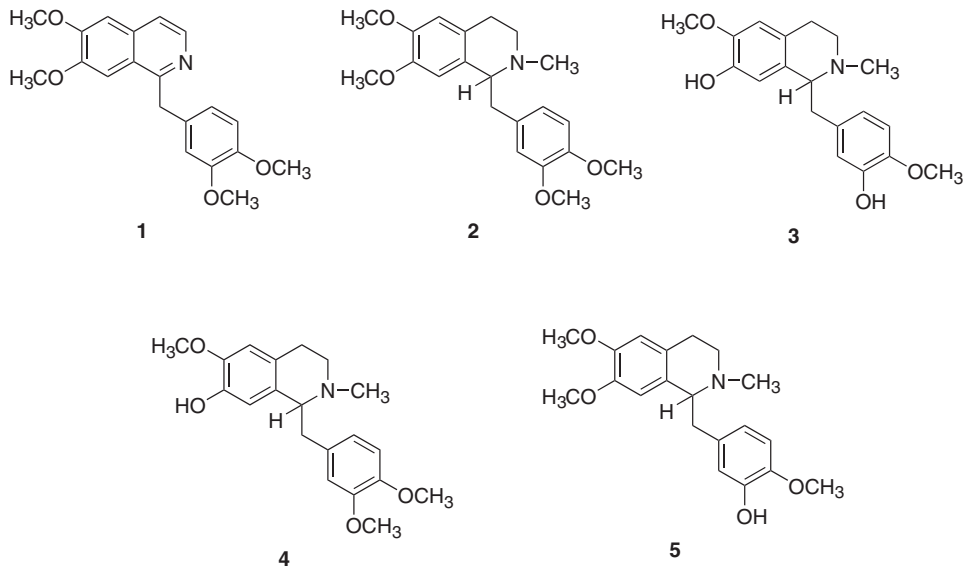


FIG. 1—*Tetrahydrobenzylisoquinoline alkaloids.*

HPLC–MS (LC/MS)

The preparative isolations were accomplished using a combination analytical/preparative HPLC Fraction Lynx system featuring a Waters (Milford, MA) 2525 binary gradient pump with flow rates from 1 to 150 mL/min. Method optimization was performed in the analytical mode at 1 mL/min using an analytical column (Phenomenex, Torrance, CA, SYNERGI MAX-RP, 150 mm × 4.6 mm, 4 μm), and then switched over to the preparative mode at 20 mL/min using a matched preparative column (Phenomenex, Torrance, CA, SYNERGI MAX-RP, 150 mm × 21.2 mm, 10 μm). The Fraction Lynx system also consisted of a Waters column fluidics organizer coupled to a Waters 2767 sample manager, a Waters 515 makeup pump, a Waters 2996 photodiode array detector, and a Waters ZQ single quadrapole mass spectrometer equipped with an APCI source. Mass Lynx V4.0 software was used to operate the system.

GC/MS

An Agilent Model 5973N quadrapole mass-selective detector (MSD) (Wilmington, DE) interfaced with an Agilent 6890N GC was used under the following parameters: the MSD was operated in the electron ionization (full scan) mode with an ionization potential of 70 eV, an electron multiplier voltage of 1447, and a scan speed of 1.34 scans/s with a low mass setting of 34 and a high mass setting of 600. The GC was fitted with a 30 m × 0.25 mm ID fused-silica capillary column coated with DB-1 film (0.25 μm). The oven temperature was programmed as follows: initial temperature, 150°C; no initial hold, (ramp 1) program rate, 4°C/min, 10 min hold, (ramp 2) program rate, 2°C/min, 2 min hold, (ramp 3) 8°C/min to final temperature, 310°C; and final hold, 6.0 min. Injector and detector temperatures were maintained at 250°C and 280°C, respectively. The inlet was set in the split mode (21.5:1) with helium as the carrier gas at 1.2 mL/min.

NMR Analysis

^{1}H and ^{13}C NMR analysis was performed using a Varian Inova 600 MHz instrument (Palo Alto, CA) using either a Varian z-Spec 5-mm broadband, pulse field gradient (PFG), variable temperature probe at 25°C or a Varian 3-mm triple resonance PFG probe at 25°C. Standard one- and two-dimensional experiments were performed for structural elucidation purposes. Spec Manager and Structure Elucidator software from Advanced Chemistry Development Inc. (Toronto, Canada) were used in making assignments.

High-Resolution Mass Spectrometry

Exact mass data were collected using an Agilent 6890N (Wilmington, DE) GC coupled to a Micromass GCT (Waters Corp., Milford, MA) time-of-flight mass spectrometer operated in EI mode recording centroid data at 3 Hz. High-purity helium was used as the GC carrier gas. Samples were prepared at approximately 100 ppm in methanol or chloroform. GC injections of 2 μL in pulsed splitless mode provided assurance of chemical purity and sufficient ion abundance for accurate mass measurement. Tris-(trifluoromethyl)-triazine was used to calibrate the instrument and to provide continuous known reference ions. The 189.9966 *m/z* ion of tris-(trifluoromethyl)-triazine was used most frequently for lock-mass calibration. Research samples were bracketed by analyses of a standard drug mix to ensure instrument performance within a few mDa as specified by the manufacturer.

Synthesis and Isolation

Laudanosine—An *in situ* two-step procedure as follows was used to prepare laudanosine (15): in a 250 mL round-bottomed flask, formalin (37%, 1 mL, 13.4 mmol) was added to a solution of tetrahydropapaverine base (2.83 g, 8.3 mmol) in 25 mL of MeOH. The mixture was stirred at room temperature for 2 h. Sodium borohydride (340 mg, 9.1 mmol) was then added slowly. After an initial foaming, the reaction was stirred at room temperature for 2 h. The reaction was quenched with 15% sodium hydroxide (10 mL), stirred for 5 min, and then extracted with chloroform (2 × 250 mL). The chloroform layer was dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to obtain 2.47 g (83%) of laudanosine base as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.64 (dd, J = 8.0, 1.8 Hz, 1H), 6.60 (d, J = 1.8 Hz, 1H), 6.56 (s, 1H), 6.06 (s, 1H), 6.75 (d, J = 8.0 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.70 (dd, J = 7.5, 5.2 Hz, 1H), 3.58 (s, 3H), 3.18 (dd, J = 9.0, 5.1 Hz, 1H), 3.14 (dd, J = 13.0 Hz, 4.8 Hz,

1H), 2.83 (m, 1H), 2.78 (m, 1H), 2.76 (m, 1H), 2.58 (dt, $J = 16.0$, 4.5, 4.5 Hz, 1H), 2.55 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.6, 147.3 (× 2), 146.3, 132.5, 129.2, 126.0, 121.9, 113.0, 111.2, 111.3, 111.0, 64.9, 55.9, 55.8 (× 2), 55.6, 47.0, 42.7, 40.9, 25.6; GC/MS (EI) *m/z* (rel. intensity) 206 (M-151,100), 190 (45), 162 (13).

Codamine and Laudanine—To a 250-mL round-bottomed flask equipped with a condenser, laudanosine base (6.67 g, 19.0 mmol) was added along with 150 mL of acetonitrile. Trimethylsilyliodide (5.2 mL, 38.0 mmol) was added dropwise, and the mixture was stirred at room temperature for 24 h. The resulting solution was concentrated *in vacuo* to obtain 10.2 g of a crude dark brown solid. The crude sample was divided and half was used for isolation of the desired bases. The crude solid (5.1 g) was dissolved in chloroform (200 mL) and washed with 1 N sulfuric acid (100 mL). The resulting aqueous layer was basified with sodium bicarbonate to pH = 10. This solution was washed with chloroform (2 × 200 mL). The chloroform layer was washed with a saturated brine solution (2 × 100 mL), dried with sodium sulfate, filtered, and concentrated *in vacuo* to obtain a gold-colored oil (1.0 g) as a complex mixture containing codamine and laudanine.

The crude reaction material (1.0 g), described above, was subjected to preparative LC/MS for isolation of codamine and laudanine. This was accomplished using a mass selective approach, where fraction collection was triggered on the molecular weight (MW 344, M+H) of the desired compounds. A single ion monitoring method (SIM) was incorporated to detect the targeted compounds only, enhancing the sensitivity for detection of the desired compounds. As the synthetic method creating the desired compounds was not selective, several (M+H, 344) compounds were present in the mixture. The combination isocratic, followed by a gradient, reversed-phase preparative HPLC method was used to separate and isolate the desired compounds, as described in Table 1. The sample was prepared by dissolving the 1 g sample in 10 mL of 1:1 acetonitrile/0.1% trifluoroacetic acid (TFA) aqueous solution. The method was set in an automated mode with multiple 500 µL injections followed by mass targeted collection. This technique provided several fractions containing the desired compounds. Codamine was collected at a retention time of 34.9 min and laudanine was collected at 44.9 min. All like fractions were combined, basified with saturated sodium bicarbonate, and extracted with chloroform. The chloroform layer was dried with sodium sulfate and concentrated to provide golden-colored glasses: codamine (22 mg) and laudanine (10 mg).

Codamine: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 (d, $J = 7.9$ Hz, 1 H), 6.73 (d, $J = 1.5$ Hz, 1H), 6.66 (dd, $J = 7.9$, 1.5 Hz, 1H), 6.63 (s, 1H), 6.18 (s, 1H), 4.19 (bd, $J = 7.0$ Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.83 (m, 1H), 3.55 (m, 1H), 3.26 (m, 1H), 3.14 (m, 1H), 3.01 (m, 2H), 2.83 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.0, 148.3, 147.0, 144.6, 127.6, 122.0, 120.4, 122.4, 114.2, 113.2, 111.2, 110.6; 65.5, 56.0 (× 2), 55.8, 45.2, 41.1, 40.6, 21.6; GC/MS (EI) *m/z* (rel. intensity) 264 (M-151+TMS, 100), 234 (15).

TABLE 1—*Preparative HPLC method used to isolate codamine and laudanine.*

Time (min)	Flow (mL/min)	%*	%†
0	20	88	12
40	20	88	12
45	20	75	25
46	20	1	99
49	20	1	99
50	20	88	12
55	20	88	12

*0.1% TFA aqueous solution.
†Acetonitrile.
HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid.

Laudanine: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.70 (d, $J = 8.2$ Hz, 1H), 6.63 (bs, 1H), 6.57 (bd, $J = 8.2$, 1H), 6.57 (s, 1H), 5.74 (bs, 1H), 4.16 (m, 1H), 4.08 (m, 1H), 3.80 (s, 6H), 3.71 (m, 1H), 3.42 (s, 3H), 3.36 (m, 1H), 3.08 (m, 2H), 2.78 (s, 3H), 2.76 (d, $J = 10.1$ Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.2, 147.3, 146.2, 145.7, 128.9, 121.9, 121.4, 120.8, 116.2, 111.1, 111.0, 110.8; 65.6, 56.0, 55.9, 55.5, 44.8, 41.3, 40.1, 21.5; GC/MS (EI) *m/z* (rel. intensity) 206 (M-137, 100), 190 (9).

Reticuline—The reticuline obtained from Tasmanian Alkaloids Inc. (Lot 182) was 70% pure; therefore, further purification was achieved using the Waters Fraction Lynx LC/MS preparative system. The 20 mL/min HPLC method consisted of an isocratic system using 76% A (0.1% TFA aqueous solution) and 24% B (acetonitrile) that produced a reticuline retention time of 10.0 min. Reticuline was isolated in milligram quantities using the same extraction protocol described earlier for the codamine and laudanine isolation.

Reticuline: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.78 (d, $J = 1.6$ Hz, 1H), 6.74 (d, $J = 8.1$ Hz, 1H), 6.61 (dd, $J = 8.2$, 1.6 Hz, 1 H), 6.56 (s, 1H), 6.36 (bs, 1H), 3.87 (m, 3H), 3.86 (s, 3H), 3.75 (bm, 1H), 3.24 (bm, 1H), 3.14 (bm, 1H), 2.83 (m, 1H), 2.82 (dd, $J = 15.2$, 5.5 Hz, 1H), 2.81 (dd, $J = 14.0$, 6.4 Hz, 1H); 2.64 (bd, $J = 14$ Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.4 (× 2), 145.1, 143.5, 132.5, 129.5, 126.3, 121.0, 115.7, 113.8, 110.6, 110.5; 64.5, 55.9, 55.8, 46.6, 42.2, 40.9, 24.7; GC/MS (EI) *m/z* (rel. intensity) 264 (M-137+TMS, 100), 234 (13).

Synthesis and Isolation of Neutral Heroin Impurities

To prove that the target neutral heroin impurities were derived from their corresponding opium natural product, individual solutions of laudanosine, codamine, laudanine, and reticuline (4 mg/mL) in acetic anhydride were placed in 15 mL disposable centrifuge tubes, sealed, and heated for up to 24 h at 115°C. After heating, small aliquots were removed and placed in a 1:1 chloroform/MSTFA solution for GC/MSD analysis. This analysis resulted in the detection of the fully acylated impurities **6–13**.

Compounds **6** and **7** were also isolated from a bulk South American heroin exhibit using mass targeted preparative LC/MS methodology, similar to the alkaloid isolation protocol mentioned earlier. Compounds **7–9** and **11–13** were isolated from the crude acetic anhydride reaction mixtures using mass targeted preparative LC/MS methodology. Compound **10** was obtained without preparative HPLC purification.

A typical HPLC method used to isolate the neutral compounds followed a 20 mL/min protocol using a reversed-phase preparative column (Phenomenex, SYNERGI MAX-RP, 150 mm × 21.2 mm, 10 µm). Either a gradient or isocratic method with a binary acetonitrile and water mixture was used. Milligram quantities of compounds **7–9** and **11–13** were collected and lyophilized to off-white powders for NMR analysis. Compounds **11–13** isomerized to a 1:1 mixture of *cis*- and *trans*-stilbene compounds during a lyophilization process. A second LC/MS purification protocol was needed to secure the *trans*-isomers from the *cis*-isomers for final elucidation. The isomerization was avoided in the second purification by keeping the isolated material covered and out of the light during the lyophilization process. The compounds also existed as a mixture of rotational isomers in solution during NMR analysis; therefore, a major and a minor rotamer were established.

Compound **6**: Major rotamer, ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.90 (s, 1H), 6.75 (d, $J = 7.6$ Hz, 1H), 6.69–6.71 (m, $J = 1.5$ Hz, 1H), 6.67 (dd, $J = 7.5$, 1.8 Hz, 1H), 6.62 (s, 1H), 6.10 (dd, $J = 7.9$, 6.2 Hz, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 2.91–3.52 (m, 4H), 2.87 (s, 3H), 2.79–2.53 (m, 2H), 2.01 (s, 3H) 1.99 (s, 3H).

Minor rotamer, ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.94 (s, 1H), 6.75 (d, J = 8.10 Hz, 1H), 6.65 (dd, J = 8.1, 2.15 Hz, 1H), 6.50 (d, J = 2.1 Hz, 1H), 6.47 (s, 1H), 5.99 (t, J = 7.1 Hz, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.69 (s, 3H), 3.48–2.91 (m, 4H), 2.86 (s, 3H), 2.51–2.82 (m, 2H), 2.02 (s, 3H), 1.83 (s, 3H); LC/MS *m/z* (mass target ion) 482 (M+Na).

Compound **7**: Major rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.10 (s, 1H), 6.89 (dd, J = 8.2, 2.0 Hz, 1H), 6.83 (d, J = 2.3 Hz, 1H) 6.83 (d, J = 8.2 Hz, 1H), 6.76 (s, 1H), 6.10 (t, J = 7.0 Hz, 1H), 3.82 (s, 3H) 3.80 (s, 3H) 3.47 (ddd, J = 13.5, 9.8, 5.8 Hz, 1H), 3.41 (ddd, J = 13.5, 9.6, 6.4 Hz, 1H), 3.08 (dd, J = 13.9, 7.5 Hz, 1H), 2.97 (dd, J = 13.9, 6.7 Hz, 1H), 2.87 (s, 3 H), 2.79 (ddd, J = 13.5, 9.6, 6.4 Hz, 1H), 2.69 (ddd, J = 13.9, 9.5, 5.5 Hz, 1H), 2.33 (s, 3H), 2.30 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 170.1, 168.9 (× 2), 150.5, 149.9, 139.4, 138.5, 135.4, 130.5, 129.2, 127.7, 124.1, 121.0, 113.6, 112.3, 72.1, 56.0, 55.9, 48.7, 42.1, 36.7, 29.9, 22.0, 21.2, 20.7, 20.7.

Minor rotamer; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (s, 1H), 6.89 (dd, J = 8.1, 2.2 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.53 (s, 1H), 5.99 (t, J = 7.2 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.43 (ddd, J = 15.0, 9.5, 6.4 Hz, 1H), 3.31 (ddd, J = 15.0, 8.7, 7.2 Hz, 1H), 3.12 (dd, J = 13.8, 6.8 Hz, 1H), 2.92 (s, 3H), 2.93 (dd, J = 13.8, 6.8 Hz, 1H), 2.65 (ddd, J = 13.80, 8.70, 6.40 Hz, 1H), 2.59 (ddd, J = 13.8, 9.0, 5.5 Hz, 1H), 2.34 (s, 3H), 2.29 (s, 3H), 2.04 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 170.2, 168.8 (× 2), 150.6, 150.1, 139.5, 139.0, 134.6, 130.4, 128.8, 127.7, 124.0, 121.3, 113.4, 112.2, 72.2, 56.0, 55.9, 51.3, 42.2, 33.4, 31.0, 21.2, 21.0, 20.7, 20.6; LC/MS *m/z* (mass target ion) 538 (M+Na); HRMS, *m/z* calculated for $C_{15}H_{20}NO_5$ (M-221): 294.1341. Found 294.1339 (− 0.8 ppm).

Compound **8**: Major rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (s, 1H), 6.74 (d, J = 8.4 Hz, 1H), 6.73 (s, 1H), 6.65 (dd, J = 8.2, 1.5 Hz, 1H), 6.54 (d, J = 1.3 Hz, 1H), 6.12 (t, J = 7.2 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H), 3.43 (ddd, J = 13.6, 9.5, 5.8 Hz, 1H), 3.28 (ddd, J = 13.8, 9.7, 6.3 Hz, 1H), 3.12 (dd, J = 13.5, 7.0 Hz, 1H), 2.98 (dd, J = 13.8, 7.7 Hz, 1H), 2.85 (s, 3H), 2.72 (ddd, J = 13.6, 9.6, 6.2 Hz, 1H), 2.64 (ddd, J = 13.7, 9.6, 5.7 Hz, 1H), 2.34 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 170.1, 168.9, 150.5, 148.7, 147.8, 138.6, 135.6, 130.7, 129.1, 121.6, 121.1, 113.5, 112.9, 111.0, 72.4, 56.0, 55.9, 55.8, 48.7, 42.7, 36.6, 29.9, 21.9, 21.2, 20.7.

Minor rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20 (s, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.68 (dd, J = 8.2, 1.5 Hz, 1H), 6.50 (s, 1H), 6.33 (d, J = 1.3 Hz, 1H), 6.00 (dd, J = 7.9, 6.9 Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 3.69 (s, 3H), 3.42 (ddd, J = 14.9, 8.6, 6.0 Hz, 1H), 3.23 (ddd, J = 14.9, 8.1, 7.2 Hz, 1H), 3.16 (dd, J = 13.4, 6.4 Hz, 1H), 2.95 (dd, J = 12.8, 7.9 Hz, 1H), 2.91 (s, 3H), 2.56 (ddd, J = 14.26, 8.40, 7.20 Hz, 1H), 2.52 (ddd, J = 14.3, 8.9, 5.4 Hz, 1H), 2.34 (s, 3H), 2.06 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.2, 168.8, 150.7, 148.6, 147.9, 139.0, 134.8, 130.6, 128.6, 121.5, 121.5, 113.2, 112.8, 111.0, 72.4, 56.0, 55.8, 55.7, 51.3, 42.7, 33.4, 31.0, 21.2, 21.0, 20.7; LC/MS *m/z* (mass target ion) 510 (M+Na); HRMS, m/z calculated for $C_{15}H_{20}NO_5$ (M-193): 294.1341. Found 294.1347 (+1.9 ppm).

Compound **9**: Major rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) 6.90 (s, 1H), 6.89 (dd, J = 6.3, 2.6 Hz, 1H), 6.84 (s, 1H), 6.82 (d, J = 1.9 Hz, 1H), 6.65 (d, J = 6.5 Hz, 1H), 6.12 (t, J = 6.9 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.80 (s, 3H), 3.48 (ddd, J = 13.6, 9.7, 5.8 Hz, 1H), 3.41 (ddd, J = 13.6, 9.4, 6.6 Hz, 1H), 3.10 (dd, J = 13.8, 7.2 Hz, 1H), 3.01 (dd, J = 13.8, 6.5 Hz, 1H), 2.87 (s, 3H), 2.76 (ddd, J = 13.8, 9.3, 6.5 Hz, 1H), 2.67 (ddd, J = 13.9, 9.2, 5.9 Hz, 1H), 2.30 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 170.1, 168.9, 149.9, 148.5, 147.8, 139.4, 130.0, 129.4, 129.0, 127.8, 124.1, 112.7, 112.2, 109.4, 72.8, 56.0, 55.9, 55.9, 48.9, 42.1, 36.7, 29.6, 21.2, 21.1, 20.7.

Minor rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) 6.89 (dd, J = 7.4, 2.8 Hz, 1H), 6.84 (d, J = 2.8 Hz, 1H), 6.82 (s, 1H), 6.65 (d, J = 7.4 Hz, 1H), 6.45 (s, 1H), 6.01 (t, J = 7.0 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.45 (ddd, J = 15.0, 9.0, 6.0 Hz, 1H), 3.30 (ddd, J = 15.0, 7.8, 7.6 Hz, 1H), 3.13 (dd, J = 14.0, 7.0 Hz, 1H), 2.96 (dd, J = 13.6, 7.4 Hz, 1H), 2.92 (s, 3H), 2.61 (ddd, J = 14.1, 9.0, 7.0 Hz, 1H), 2.56 (ddd, J = 14.1, 8.6, 5.8 Hz, 1H), 2.29 (s, 3H), 2.06 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) 170.6, 170.2, 168.8, 150.0, 148.7, 148.2, 139.5, 129.9, 129.1, 128.4, 127.7, 124.0, 112.5, 112.2, 109.5, 72.8, 56.1, 56.0, 55.9, 51.6, 42.2, 33.5, 30.7, 22.0, 21.2, 20.6; LC/MS *m/z* (mass target ion) 510 (M+Na).

Compound **10**: Major rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, J = 16.1 Hz, 1H), 7.30 (d, J = 1.5 Hz, 1H), 7.16 (s, 1H), 7.08 (dd, J = 8.0, 1.6 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.88 (d, J = 16.1 Hz, 1H), 6.70 (s, 1H), 4.03 (s, 3H), 3.96 (s, 3H), 3.91 (s, 6H), 3.53 (dd, J = 8.0, 7.0 Hz, 2H), 2.99 (dd, J = 8.5, 6.4 Hz, 2H), 2.83 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 149.3, 148.7, 148.6, 147.9, 130.9, 129.7, 129.4, 127.9, 123.6, 119.8, 113.0, 111.2, 108.6, 108.0, 56.1, 56.0 (× 2), 55.9, 50.3, 37.4, 30.9, 22.0.

Minor rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12 (d, J = 16.1 Hz, 1H), 7.10–7.09 (m, 1H), 7.07 (d, J = 6.5, 1.5 Hz, 1H), 7.03 (d, J = 1.5 Hz, 1H), 6.89 (d, J = 6.8 Hz, 3H), 6.87 (d, J = 16.0 Hz, 1H), 6.60 (s, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.48 (t, J = 7.2 Hz, 2H), 2.97 (s, 3H), 2.96 (t, J = 7.6 Hz, 5H), 1.90 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 149.2, 149.0, 148.8, 148.2, 130.5, 129.1, 129.0, 128.4, 123.3, 119.4, 113.1, 111.4, 109.0 (× 2), 56.1, 56.0 (× 2), 55.9, 52.0, 33.6, 32.1, 21.0.

Compound **11**: Major rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) 7.38 (dd, J = 8.4, 2.0 Hz, 1H), 7.28 (s, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.24 (d, J = 16.0 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.80 (s, 1H), 6.80 (d, J = 16.0 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.55 (dd, J = 8.5, 6.4 Hz, 2H), 3.00 (dd, J = 8.4, 6.7 Hz, 2H), 2.84 (m, 3H), 2.35 (s, 3H), 2.33 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 169.0, 169.0, 150.6, 150.4, 140.0, 138.7, 135.7, 130.9, 129.5, 128.1, 125.2, 124.0, 123.6, 119.7, 113.9, 112.5, 56.0, 55.9, 49.7, 37.4, 31.2, 22.0, 20.7, 20.7.

Minor rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (dd, J = 8., 2.0 Hz, 1H), 7.18 (d, J = 2.1 Hz, 1H), 7.05 (d, J = 16.0 Hz, 1H), 6.96 (d, J = 8.1 Hz, 1H), 6.81 (d, J = 16.0 Hz, 1H), 6.67 (s, 1H), 3.86 (s, 2H), 3.85 (s, 3H), 3.51 (dd, J = 8.3, 5.9 Hz, 2H), 2.99–2.95 (m, J = 8.5, 6.0 Hz, 3H), 2.35 (s, 3H), 2.33 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 169.0, 168.9, 150.9, 150.6, 139.4, 139.0, 134.4, 130.9, 128.9, 127.7, 125.3, 124.0, 123.0, 120.5, 113.9, 112.5, 56.0 (× 2), 51.6, 33.4, 32.4, 20.9, 20.6, 20.6; LC/MS *m/z* (mass target ion) 478 (M+Na), HRMS, *m/z* calculated for $C_{25}H_{29}NO_7$ (molecular ion): 455.1944. Found 455.1961 (+3.7 ppm).

Compound **12**: Because of isomerization during isolation, material was only available for GC/MSD analysis; LC/MS *m/z* (mass target ion) 450 (M+Na).

Compound **13**: Major rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (dd, J = 8.5, 2.1 Hz, 1H), 7.30 (d, J = 2.1 Hz, 1H), 7.29 (d, J = 16.0 Hz, 1H), 7.11 (s, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.84 (d, J = 16.1 Hz, 1H), 6.71 (s, 1 H), 3.95 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H), 3.54 (t, J = 7.3 Hz, 2H), 2.98 (t, J = 7.5 Hz, 2H), 2.81 (s, 3H), 2.36 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 169.1, 150.5, 148.8, 147.9, 140.0, 131.1, 129.9, 128.8, 127.1,

125.0, 124.5, 120.7, 113.1, 112.6, 108.2, 56.0 (× 3), 50.0, 37.4, 30.8, 22.0, 20.7.

Minor rotamer, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (dd, J = 8.4, 1.9 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.11 (d, J = 16.1 Hz, 1H), 7.08 (s, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 16.1 Hz, 1H,) 6.59 (s, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.47 (t, J = 7.2 Hz, 2H), 2.96 (s, 3H), 2.95 (t, J = 7.2 Hz, 2H), 2.36 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.0, 150.7, 148.9, 148.3, 140.0, 130.7, 128.7, 128.5, 128.3, 125.1, 124.0, 120.3, 113.1, 112.5, 108.9, 56.1, 56.0 (× 2), 52.0, 33.6, 32.0, 20.9, 20.7; LC/MS *m/z* (mass target ion) 450 (M+Na).

Hydrolysis of Neutral Heroin Impurities

Neutral heroin impurities, compounds (**7–9** and **11–13**), were individually placed in 15 mL disposable centrifuge tubes containing 250 µL of 3 N HCl in methanol. The tubes were sealed and heated for 15 min at 55°C. The solutions were concentrated to light brown oils. These oils were taken up in a 1:1 chloroform/MSTFA solution for GC/MSD analysis.

Results and Discussion

In order to better understand the degradation pathway and rationalize the presence of these new impurities in heroin, the synthesized or isolated tetrahydrobenzylisoquinolines were individually treated with acetic anhydride and heated. The results demonstrate that these compounds undergo a facile and consistent degradation process ending in neutral impurity formation (16).

The mechanism for the process of tertiary amine reaction with acetic anhydride has been debated in the past (17–19). This debate has been between traditional S_N1-vs S_N2-type proposals. Another

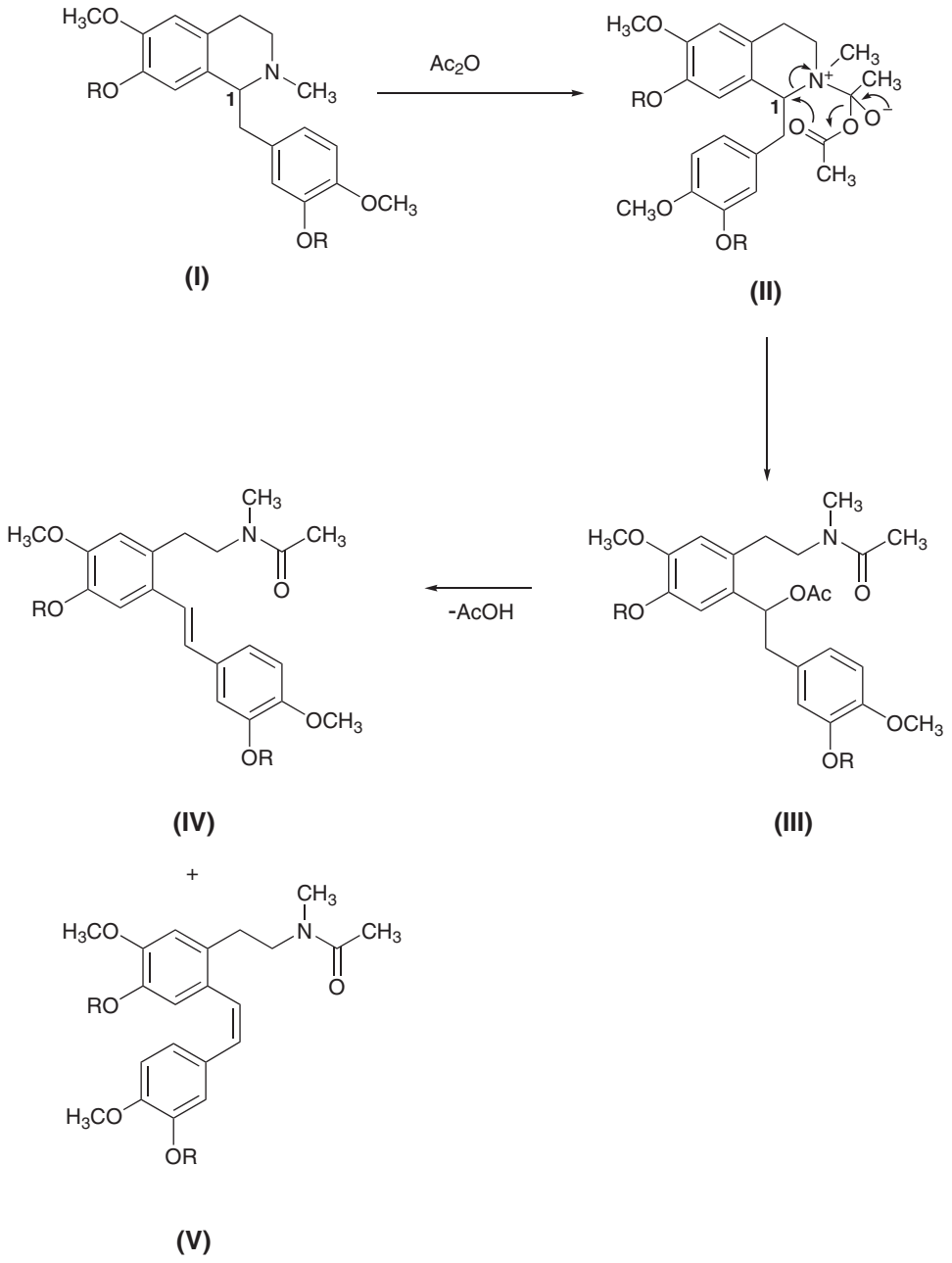


FIG. 2—*Mechanism for neutral impurity formation.*

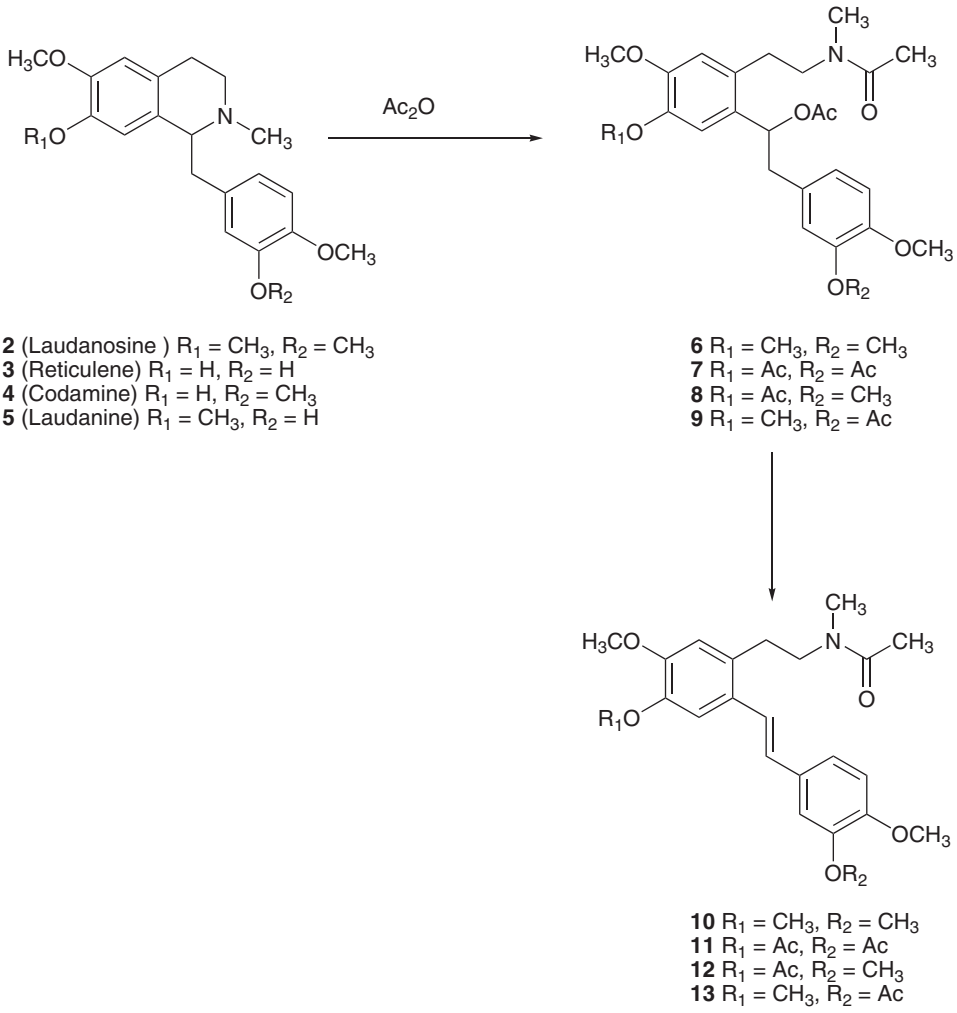
possible mechanism involves a concerted type process, where formation of the amide and the acetate formation at C-1 occur concurrently. This process starts with reaction of the tertiary amine nitrogen located in the B ring of the isoquinoline moiety I with acetic anhydride (Fig. 2). This reaction generates activated intermediate II, which undergoes a six-centered intramolecular rearrangement producing the C-1 substituted acetate impurity III. Impurity III then undergoes elimination of acetic acid to the more thermally stabile *trans*-stilbene impurity IV and *cis*-stilbene V. The abundances of acetate impurity III and *trans*-stilbene impurity IV are dependent on how long the acetic anhydride reaction was allowed to proceed. In general, as the reaction time increases, the presence of impurity III decreases as impurity IV increases. The *cis*-stilbene compounds are present in heroin at such low quantities, as compared with their *trans*-stilbene counterparts, and therefore are not a subject of this paper.

Formation of Neutral Heroin Impurities in Acetic Anhydride

The first possible set of neutral heroin impurities starting from the featured tetrahydrobenzylisoquinolines alkaloids is shown in Fig. 3. These compounds follow the acetic anhydride degradation pathway described above. Laudanosine **2** degrades to acetate impurity 6, and then further to *trans*-stilbene **10** upon longer reaction time. Reticuline **3** undergoes degradation to acetate impurity **7** and *trans*-stilbene **11**, while codamine **4** undergoes degradation to acetate impurity **8** and *trans*-stilbene **12**. Laudanine **5** degrades to acetate impurity **9** and *trans*-stilbene **13**.

The phenomenon of acetic anhydride degradation of reticuline, as a function of time, is showcased as ratios of acetate impurity **7** to *trans*-stilbene **11** in Table 2. This degradation study illustrates that compound **7** was maximized in the first hour of heating, while the *trans*-stilbene **11** was maximized after 8 h of heating. After 5 h of heating, there were approximately equal amounts of both products. After 24 h of heating, all of acetate impurity **7** was converted over to product **11**. Therefore, the length of the acetic anhydride heating time was a critical factor in the formation of the impurities from reticuline and the same analogy was observed for impurities starting from the other tetrahydrobenzylisoquinoline compounds as well.

Because of the labile nature of the phenolic acetate functionality present, compounds **11**, **12**, and **13** were readily hydrolyzed to a second set of impurities also present in heroin, as shown in Fig. 4. Compound **11** underwent partial hydrolysis to regioisomers **14** and **15** or complete hydrolysis to compound **16**. Analogously, compounds **12** and **13** underwent hydrolysis to compounds **17** and **18**, respectively. Complete hydrolysis of **11**, **12**, and **13** producing **16–18** was accomplished using **3** N HCl in methanol. The milder hydrolysis products **14** and **15** were generated as a result of compound **11** undergoing degradation in storage.



2 (Laudanosine) $R_1 = CH_3$, $R_2 = CH_3$
3 (Reticulene) $R_1 = H$, $R_2 = H$
4 (Codamine) $R_1 = H$, $R_2 = CH_3$
5 (Laudanine) $R_1 = CH_3$, $R_2 = H$

6 $R_1 = CH_3$, $R_2 = CH_3$
7 $R_1 = Ac$, $R_2 = Ac$
8 $R_1 = Ac$, $R_2 = CH_3$
9 $R_1 = CH_3$, $R_2 = Ac$

10 $R_1 = CH_3$, $R_2 = CH_3$
11 $R_1 = Ac$, $R_2 = Ac$
12 $R_1 = Ac$, $R_2 = CH_3$
13 $R_1 = CH_3$, $R_2 = Ac$

FIG. 3—*Reaction of tetrahydrobenzylisoquinolines with acetic anhydride.*

TABLE 2—*Degradation of reticuline in acetic anhydride.*

Time (h)	Ratio of 7/11
1	5.7/1.0
2	4.7/1.0
3	2.7/1.0
4	1.8/1.0
5	1.1/1.0
6	1.0/1.6
7	1.0/3.0
8	1.0/3.3

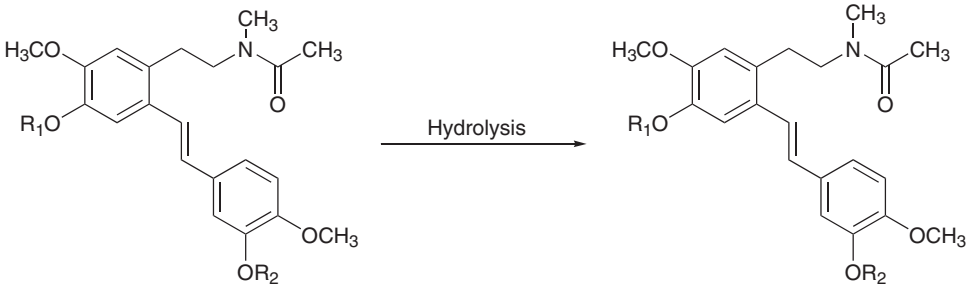
A third set of impurities resulted from mild or partial hydrolysis of acetate impurities **7**, **8**, and **9**, as shown in Fig. 5. Because of the unstable nature of these materials, the mild hydrolysis impurity products were monitored as a function of degradation of its precursor over time. For example, compound **7** underwent partial hydrolysis to regioisomers **19** and **20** or to the further hydrolytic compound **21** upon standing over time. Compounds **8** and **9** also underwent mild hydrolysis over time to **22** and **23**, respectively. An attempt to hydrolyze compounds **7**–**9** with **3** N HCl resulted in the elimination of acetic acid forming the stilbene compounds **16**, **17**, or **18**, respectively.

Characterization of Neutral Impurities

All impurities were monitored using GC/MSD methodology, as described in the "Experimental." The GC experiments were performed in the presence of MSTFA to provide, in some cases, trimethylsilyl derivatives (TMS). This reagent provided stability for the thermally unstable acetate impurities shown in Fig. 5, and it also provided results identical to GC/MSD analytical data generated by the heroin signature program. The resulting molecular weights and retention times for the new impurities, along with electron impact fragmentation results, are shown in Table 3.

The mass spectra for impurities **6**–**9**, shown in Fig. 6, indicated that the molecular ion showed a very weak response. This result was because of the rapid thermal elimination of acetic acid forming stilbene compounds. As a result, the stilbene fragment, [M-60], was a common ion. The key base peaks for these four compounds were either *m/z* 266 **24** or *m/z* 294 **25**, as shown in Fig. 7. A plausible fragmentation pathway responsible for these ions starts with a radical cation on the nitrogen atom of the *N*-methylacetamide side chain. This reactive species can undergo an intramolecular-mediated proton transfer and subsequent loss of ketene followed by benzylic cleavage to obtain fragment **25**. Gas chromatography high-resolution mass spectrometry, performed on compounds **7** and **8**, produced the *m/z* 294 ion and confirmed the $C_{15}H_{20}NO_5$ aldehyde fragment **25**.

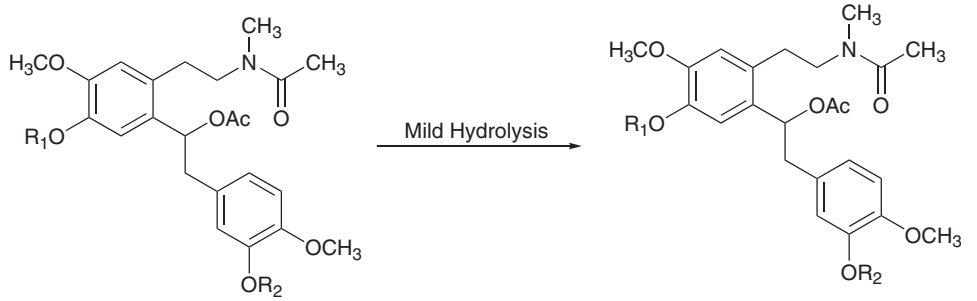
The stilbene compounds **10**–**13** produced solid molecular ion information (Fig. 8). Other key fragments, also shown in Fig. 7, included the *m/z* 151 ion **27** for impurities **10** and **12**, whereas impurities **11** and **13** produced *m/z* 179 **28** and *m/z* 137 **30** ions, as a result of the presence of a phenolic acetate in the C ring. These were critical ions for tracking compounds, because the



11 R_1 = Ac, R_2 =Ac
12 R_1 = Ac, R_2 = CH_3
13 R_1 = CH_3 , R_2 =Ac

14 R_1 = H, R_2 = Ac
15 R_1 = Ac, R_2 = H
16 R_1 = H, R_2 = H
17 R_1 = H, R_2 = CH_3
18 R_1 = CH_3 , R_2 = H

FIG. 4—*Hydrolysis of neutral impurities.*



7 R_1 = Ac, R_2 = Ac
8 R_1 = Ac, R_2 = CH_3
9 R_1 = CH_3, R_2 = Ac

19 R_1 = H, R_2 = Ac
20 R_1 = Ac, R_2 = H
21 R_1 = H, R_2 = H
22 R_1 = H, R_2 = CH_3
23 R_1 = CH_3, R_2 = H

FIG. 5—*Mild hydrolysis of neutral impurities.*

TABLE 3—*GC/MSD results for neutral impurities.*

Compound	MW	RT (min)	*m/z* (Relative Intensity)
6	459	43.2	459(0.2), 399(62), 326(35), 266(100), 151(71), 44(38)
7	515	45.8	515(1), 455(28), 294(100), 179(19), 137(23), 44(26)
8	487	44.6	487(0.2), 427(47), 294(100), 151(45), 44(26)
9	487	45.1	487(1), 427(47), 266(100), 193(44), 175(73), 44(31)
10	399	46.4	399(66), 326(45), 151(100), 44(32)
11	455	49.4	455(76), 340(86), 298(100), 179(39), 137(47), 44(64)
12*	427	47.9	427(100), 312(76), 151(80), 44(35)
13*	427	47.9	427(85), 354(100), 312(76), 179(51), 137(66), 44(54)
14†	485	48.1	485(57), 412(100), 370(48), 179(37), 137(33), 44(54)
15†	485	48.2	485(100), 370(69), 209(84), 137(43), 44(57)
16‡	515	47.0	515(70), 500(13), 442(59), 209(100), 73(39), 44(22)
17†	457	46.7	457(63), 384(59), 151(100), 73(27), 44(24)
18†	457	46.9	457(72), 384(49), 209(100), 73(19), 44(26)
19,**20**†	545,545	45.1	545(0.9), 530(7), 294(100), 233(58), 209(37), 179(31)
21‡	575	44.1	575(0.3), 560(11), 515(40), 324(100), 233(51), 209(44)
22†	517	43.4	517(0.5), 502(6), 457(62), 324(100), 233(62), 151(67)
23†	517	44.0	517(0.2), 502(8), 457(49), 266(100), 209 (50), 175(32)

*Compounds **12** and **13** were individually isolated and produced identical retention times.
†Compounds were monosilylated using MSTFA in chloroform.
‡Compounds were bissilylated using MSTFA in chloroform.
GC/MSD, gas chromatography/mass-selective detector; MW, molecular weight.

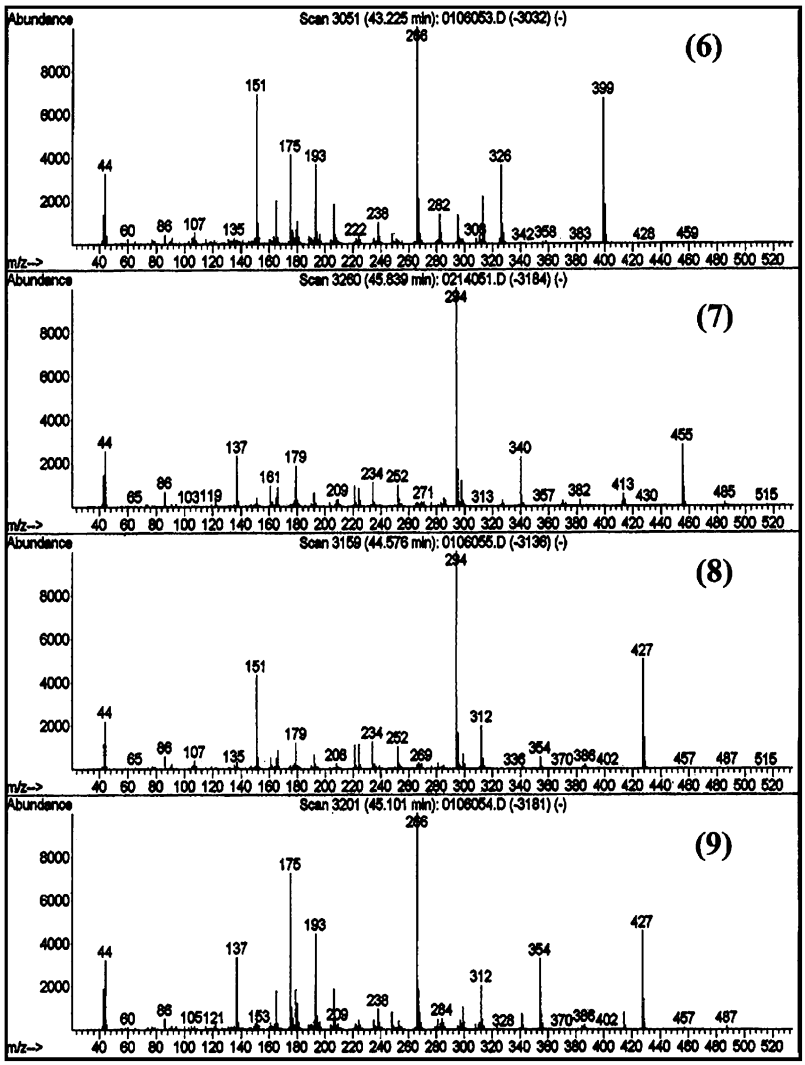
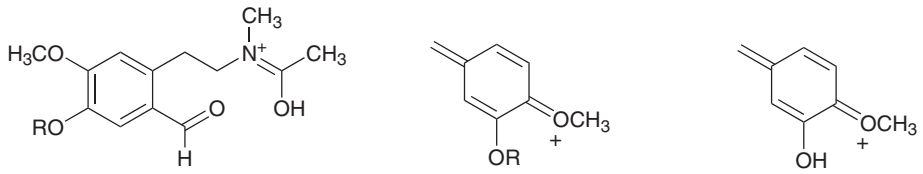


FIG. 6—*Mass spectra for neutral heroin impurities* **6–9**.



24 R = CH_3
25 R = Ac
26 R =TMS

27 R = CH_3
28 R = Ac
29 R =TMS

30

FIG. 7—*Key mass spectra fragments for neutral impurities.*

impurities resulting from laudanosine and codamine produced *m/z* 151 ions, while impurities from reticuline and laudanine produced the combination *m/z* 179 and *m/z* 137 ions.

The same fragmentation scenario holds for the hydrolyzed stilbene compounds **14**–**18**, shown in Fig. 9. Good molecular ion information was secured for each impurity. The regioisomers **14** and **15** were differentiated using the combination *m/z* 179 and *m/z* 137 ions for compound **14**, and the silylated *m/z* 209 ion **29** was an important fragment for tracking compound **15**. Compound **16** produced the expected *m/z* 209 ion, as well. Impurity **17** produced the familiar *m/z* 151 ion tracking back to a codamine impurity, while compound **18** tracked to a laudanine impurity producing the expected silylated *m/z* 209 ion as its base peak.

The mass spectra from impurities **19**–**23**, shown in Fig. 10, produced a fragmentation pathway as a result of an acetate moiety at the C-1 position. Poor molecular ion information was obtained again because of the labile acetate and facile loss of acetic acid. Compounds **19** and **20** were separated by only 0.02 min by the GC column, and as a result, a combined mass spectrum was obtained. This series of compounds produced base peak ions of *m/z* 266, *m/z* 294, or the silylated *m/z* 324 ion **26**. In all cases, a loss of methyl from the trimethylsilyl group produced an M-15 ion. A common

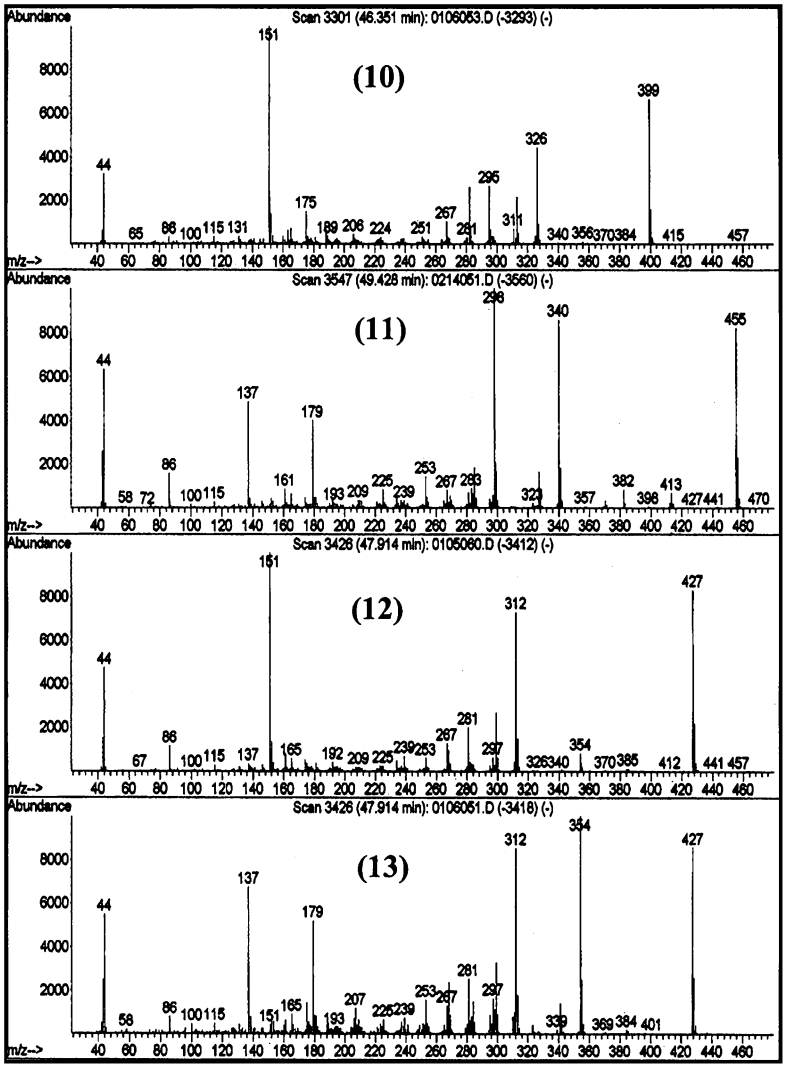


FIG. 8—*Mass spectra for neutral heroin impurities* **10**–**13**.

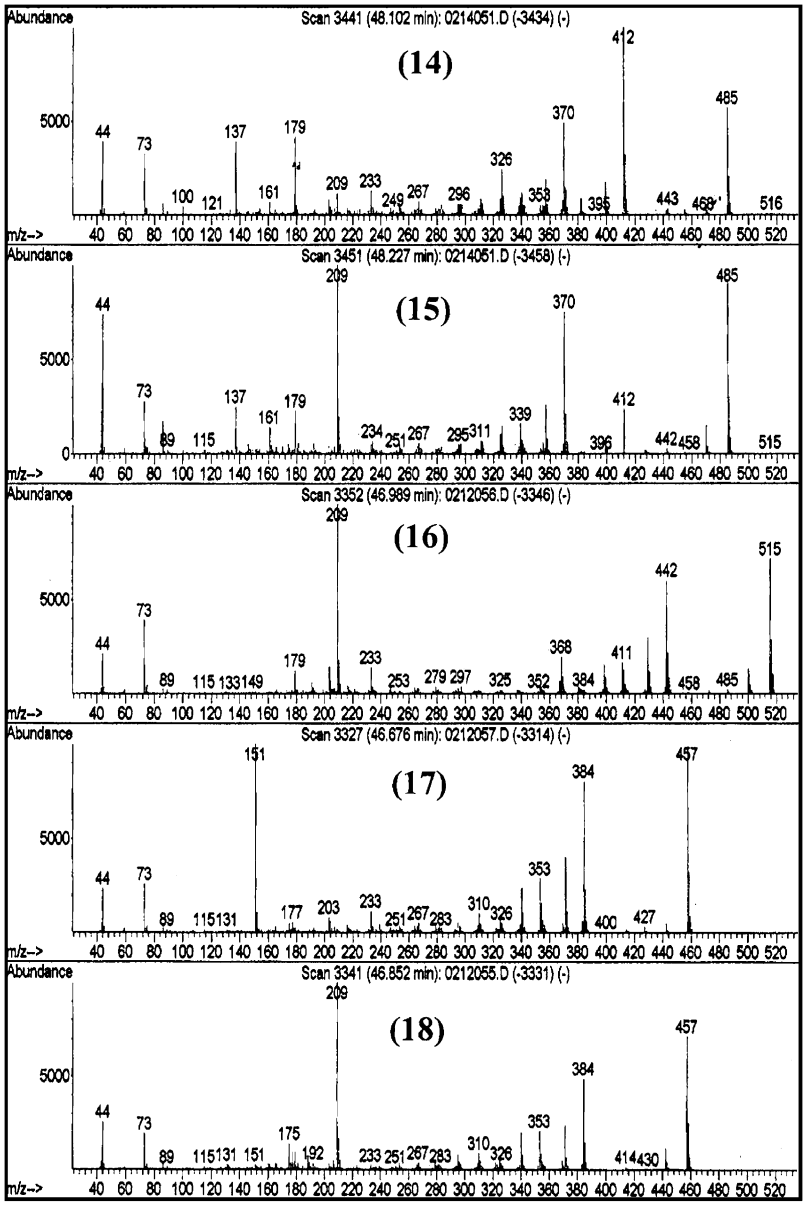


FIG. 9—*Mass spectra for neutral heroin impurities* **14**–**18**.

m/*z* 44 ion was produced for all reported impurities. This ion was the result of fragmentation of the *N*-methylacetamide side chain.

Further structural elucidation of impurities was available by analyzing the acquired NMR data for compounds **6**–**11** and **13**. In all cases, the resulting NMR spectra indicated a mixture of rotational isomers. The approximate ratio was a 1.5:1 mixture of major to minor rotamers at room temperature. These rotamers were a result of a rotational barrier existing between the *cis*-*N*-methylacetamide **31** and the *trans*-*N*-methylacetamide **32** side chains, as shown in Fig. 11. The *cis*-rotamer was predominant at room temperature, and the two were distinguished using a nuclear Overhauser experiment (nOe). The *cis*-rotamer showed a nnOe correlation from the *N*-methyl to the acetamide methyl, while there was no nOe detected for the *trans*-rotamer.

The structures for the C-1 acetate compounds **6**–**9** were supported with two-dimensional NMR data. A critical proton spin system was identified in each compound from COSY data. A correlation existed between the methine proton at C-1 (6 ppm) and the methylene protons at C-9 (3 ppm). This same methine proton at 6 ppm also showed a key three-bond HMBC correlation to the acetate carbonyl (170 ppm) that secured the acetate in the C-1 position. Connection of this spin system to the aromatic A and C rings was made using two- and three-bond proton to carbon HMBC correlations. The *N*-methylacetamide side chain was also confirmed and connected to the aromatic A ring using the same series of two-dimensional experiments consisting of COSY, HSQC, and HMBC data.

The stilbene compounds **10**, **11**, and **13** showed similar NMR characteristics, except for the presence of two olefinic doublets assigned to the double-bond protons. Large coupling constants of approximately 16 Hz secured the *trans*-double bond assignments.

Forensic Analysis of Heroin Samples

The DEA heroin signature program relies on samples obtained from the heroin producing regions of South America (SA), South-

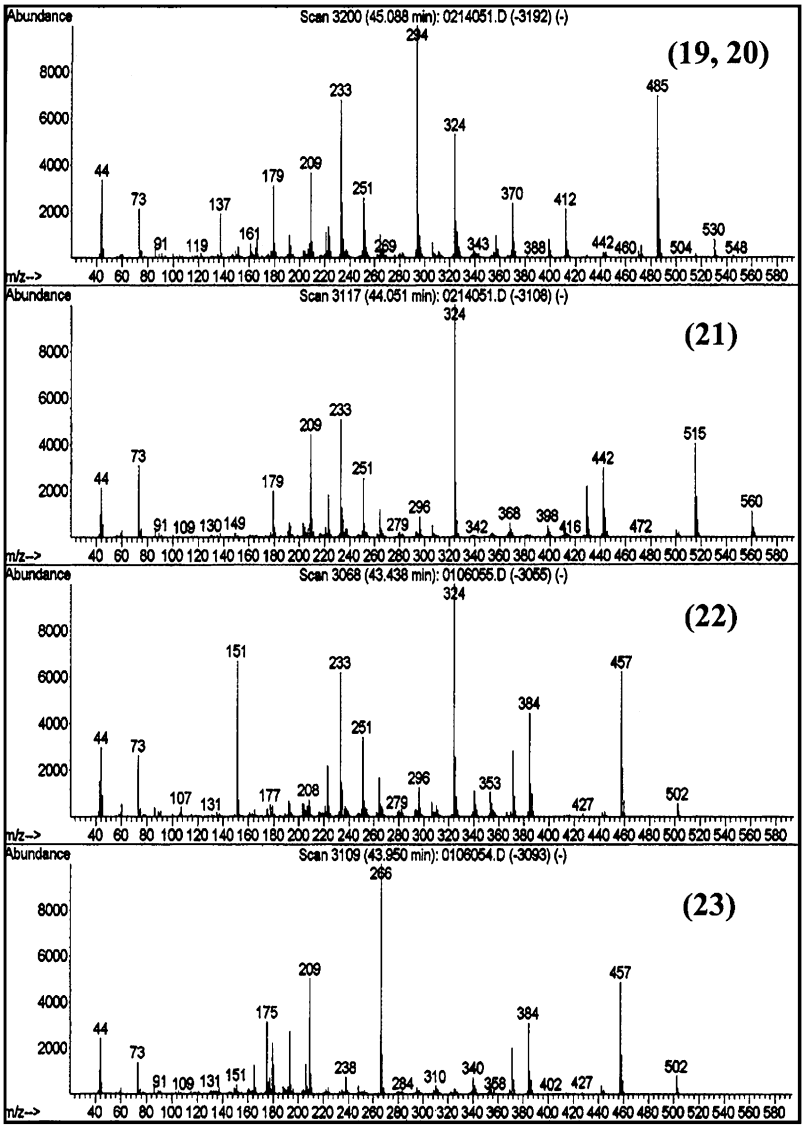


FIG. 10—*Mass spectra for neutral heroin impurities* **19**–**23**.

west Asia (SWA), Mexico (MEX), and Southeast Asia (SEA). These samples are called "authentics" and are analyzed using the established chemical signatures to produce an effective authentic collection. These authentic samples are needed to establish final classification categories for each region.

As part of the DEA heroin signature program, over 600 authentic heroin samples have been analyzed to establish a trace neutral signature database. These results, created by GC/MSD analysis, have been an instrumental part for establishing final heroin classifications. The results have warranted division of the SA

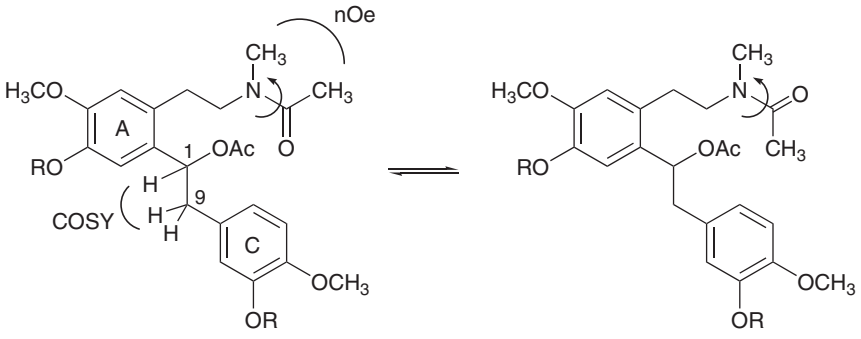


FIG. 11—*NMR analysis of rotamers.*

and SWA samples into subgroups based on overall process impurity levels. The crudest SWA heroin samples are placed into an SWA/A category, while the highly refined samples are labeled as SWA/B. Samples falling in the middle of these two purity categories are called SWA/C samples. Similar nomenclature has been established for the SA samples, where the crudest samples are labeled SA3. The highly refined samples are characterized as SA2, while SA1 samples represent the middle. The SEA samples can also be divided into subcategories based on purity; however, only one type of SEA heroin is part of this study.

The data from the authentic neutral impurity database were reprocessed to target the new impurities. New trace neutral impurities **6–18**, **22**, and **23** were tracked and quantified. Compounds **19–21** were not part of this reprocessing study. The results of the featured compounds are shown in Table 4. The results show that trace quantities of these neutral impurities are present in heroin samples, and the actual amount of each is calculated as an average percentage relative to total morphine content. Total morphine content refers to the amount of heroin and morphine related impurities converted back to morphine. A 95% confidence interval and detection percentage for all compounds in each category is also shown in Table 4.

Analysis of the SWA/A samples indicates that all 15 targeted impurities are present at the highest levels (10^{-2}) and detected nearly 100% of the time. The SWA/C samples have a similar profile but are present in the 10^{-3}–10^{-4} range. The impurities are

TABLE 4—*Summary of neutral impurities monitored in heroin samples.*

	SWA/A	SWA/B	SWA/C	SA1	SA2	SA3	MEX	SEA
#	100	25	50	88	22	173	58	164
	Ave	Ave	Ave	Ave	Ave	Ave	Ave	Ave
	95%(CI)	95%(CI)	95%(CI)	95%(CI)	95%(CI)	95%(CI)	95%(CI)	95%(CI)
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
6	2.4×10^{-3}	5.5×10^{-6}	4.0×10^{-4}	5.6×10^{-5}	2.2×10^{-5}	1.3×10^{-4}	NA	1.1×10^{-5}
	1.1×10^{-3}	4.5×10^{-6}	2.9×10^{-4}	1.9×10^{-5}	1.1×10^{-5}	6.0×10^{-5}	NA	8.4×10^{-6}
	93	32	66	74	55	54	0	4
7	7.0×10^{-2}	9.7×10^{-5}	4.1×10^{-3}	8.6×10^{-4}	2.9×10^{-4}	$5.0x10^{-3}$	3.4×10^{-3}	3.8×10^{-5}
	1.8×10^{-2}	5.2×10^{-5}	2.0×10^{-3}	3.8×10^{-4}	2.4×10^{-4}	8.6×10^{-4}	1.6×10^{-3}	2.0×10^{-5}
	95	56	70	99	27	98	35	34
8	1.1×10^{-2}	2.6×10^{-5}	1.2×10^{-3}	1.8×10^{-4}	3.9×10^{-5}	8.9×10^{-4}	1.7×10^{-3}	1.6×10^{-5}
	3.8×10^{-3}	1.5×10^{-5}	7.4×10^{-4}	4.8×10^{-5}	2.9×10^{-5}	1.4×10^{-4}	1.2×10^{-3}	9.1×10^{-6}
	100	64	80	94	59	98	33	27
9	8.2×10^{-3}	2.0×10^{-5}	8.5×10^{-4}	1.1×10^{-4}	2.6×10^{-5}	2.9×10^{-4}	3.6×10^{-3}	5.5×10^{-6}
	6.1×10^{-3}	1.5×10^{-5}	5.0×10^{-4}	4.0×10^{-5}	1.7×10^{-5}	6.8×10^{-5}	1.9×10^{-3}	3.9×10^{-6}
	100	48	82	85	82	93	24	17
10	2.0×10^{-2}	2.8×10^{-5}	1.4×10^{-3}	9.0×10^{-4}	2.5×10^{-4}	4.4×10^{-3}	1.3×10^{-2}	5.9×10^{-5}
	3.6×10^{-3}	1.2×10^{-5}	7.9×10^{-4}	3.2×10^{-4}	2.0×10^{-4}	8.2×10^{-4}	2.0×10^{-3}	3.9×10^{-5}
	99	76	74	98	91	99	100	15
11	1.3×10^{-2}	3.9×10^{-5}	2.3×10^{-3}	5.6×10^{-4}	1.8×10^{-4}	3.7×10^{-3}	4.2×10^{-2}	1.4×10^{-4}
	4.2×10^{-3}	1.6×10^{-5}	1.3×10^{-3}	2.5×10^{-4}	1.8×10^{-4}	5.7×10^{-4}	1.1×10^{-2}	8.7×10^{-5}
	98	64	56	89	32	99	97	15
12	1.3×10^{-2}	4.7×10^{-6}	3.0×10^{-4}	5.1×10^{-4}	1.3×10^{-5}	3.2×10^{-4}	3.4×10^{-2}	1.3×10^{-4}
	4.0×10^{-3}	$3.6 \times 10^{--6}$	2.4×10^{-4}	2.0×10^{-5}	1.2×10^{-5}	7.5×10^{-5}	8.8×10^{-3}	7.6×10^{-6}
	99	68	66	92	64	99	100	24
13	5.4×10^{-3}	6.3×10^{-6}	2.7×10^{-4}	5.1×10^{-4}	7.5×10^{-5}	1.6×10^{-3}	6.2×10^{-3}	4.6×10^{-5}
	1.3×10^{-3}	3.1×10^{-6}	6.2×10^{-4}	1.1×10^{-4}	9.8×10^{-5}	3.0×10^{-4}	1.6×10^{-3}	2.9×10^{-5}
	95	52	98	92	50	98	100	14
14	1.4×10^{-3}	4.4×10^{-6}	1.3×10^{-4}	1.3×10^{-4}	8.8×10^{-6}	4.5×10^{-4}	9.9×10^{-4}	5.3×10^{-5}
	1.4×10^{-3}	2.7×10^{-6}	8.0×10^{-5}	5.8×10^{-5}	9.0×10^{-6}	9.8×10^{-5}	2.1×10^{-4}	2.7×10^{-6}
	99	64	74	98	82	99	100	15
15	3.4×10^{-3}	7.6×10^{-6}	2.9×10^{-4}	2.0×10^{-4}	1.9×10^{-5}	8.1×10^{-4}	2.8×10^{-3}	1.1×10^{-5}
	3.3×10^{-3}	4.2×10^{-6}	2.0×10^{-4}	8.8×10^{-5}	1.6×10^{-5}	2.1×10^{-4}	6.2×10^{-4}	5.7×10^{-6}
	97	56	64	97	55	98	100	15
16	1.5×10^{-1}	1.5×10^{-5}	6.4×10^{-4}	1.2×10^{-3}	1.2×10^{-4}	1.7×10^{-3}	6.7×10^{-3}	1.1×10^{-5}
	6.6×10^{-2}	1.3×10^{-5}	1.0×10^{-3}	6.2×10^{-4}	1.0×10^{-4}	1.1×10^{-3}	2.6×10^{-3}	5.3×10^{-6}
	100	84	88	98	86	97	91	40
17	3.3×10^{-2}	7.1×10^{-6}	1.5×10^{-4}	1.9×10^{-4}	7.7×10^{-5}	1.0×10^{-3}	2.0×10^{-2}	8.5×10^{-6}
	1.1×10^{-2}	2.9×10^{-6}	8.1×10^{-5}	1.0×10^{-4}	9.9×10^{-5}	1.2×10^{-3}	5.8×10^{-3}	4.3×10^{-6}
	75	76	86	94	82	93	95	13
18	1.3×10^{-2}	1.9×10^{-5}	7.8×10^{-4}	9.9×10^{-4}	1.8×10^{-4}	2.7×10^{-3}	5.1×10^{-3}	3.7×10^{-5}
	5.9×10^{-3}	1.1×10^{-5}	4.8×10^{-4}	5.8×10^{-4}	2.1×10^{-4}	9.1×10^{-4}	1.2×10^{-3}	$2.1 \times 10^{--5}$
	100	68	68	98	68	96	93	11
22	6.4×10^{-3}	7.4×10^{-6}	3.6×10^{-4}	1.0×10^{-4}	5.2×10^{-5}	1.6×10^{-4}	3.1×10^{-5}	4.2×10^{-6}
	2.4×10^{-3}	8.2×10^{-6}	3.2×10^{-4}	4.4×10^{-5}	4.9×10^{-5}	3.4×10^{-5}	2.8×10^{-4}	2.3×10^{-6}
	84	32	68	65	50	53	9	11
23	1.2×10^{-2}	1.8×10^{-5}	6.4×10^{-4}	2.3×10^{-4}	8.3×10^{-5}	2.5×10^{-4}	NA	5.4×10^{-6}
	1.2×10^{-2}	1.3×10^{-5}	4.8×10^{-4}	1.1×10^{-4}	6.3×10^{-5}	9.0×10^{-5}	NA	2.9×10^{-6}
	81	28	72	61	64	54	0	10

number of authentic samples analyzed; Ave, average quantitation value relative to total morphine content; CI, confidence interval; (%), percent detected in each heroin classification; NA, not applicable.

also present in the highly purified SWA/B samples at the 10^{-5}–10^{-6} levels, but are detected less frequently. The lower frequency of detection for cleaner samples can be attributed to either the compounds being absent, or they are present at levels below the threshold of detection.

The same scenario holds for the three SA categories; however, the crude SA3 samples are approximately an order magnitude cleaner than the crude SWA/A samples. Also, the entire set of compounds is detected more frequently in the SA1 and SA3 samples, as compared with SWA/C samples with similar average quantitation values. Several key impurities are detected nearly every time for SA1 and SA3 samples. These compounds include both the C-1 acetate impurities **7–9** and the stilbene compounds **10–18**.

The Mexican profile looks completely different from other samples, where the stilbene compounds **10–18** are detected at 10^{-2}–10^{-3} levels nearly 100% of the time. The C-1 acetate impurities are either not detected at all or very infrequently. These results fit the described degradation pathway, because the C-1 acetate impurities do not survive the harsh reaction condition needed to make a Mexican "tar" sample.

The results for the SEA samples indicated that the featured impurities were detected in only a small portion of the authentic samples. The majority of the samples did not contain any of the targeted compounds because of their high heroin purity levels.

Conclusions

A chemical rationale was described for the degradation of four tetrahydrobenzylisoquinoline alkaloid impurities present in the acylation of morphine to heroin. These four impurities degraded to eighteen neutral impurities that were detected in heroin. In the presence of heated acetic anhydride, laudanosine was found to degrade to compounds **6** and **10**, while reticulene degraded to compounds **7**, **11**, **14–16**, and **19–21**. Codamine degraded to compounds **8**, **12**, **17**, and **22**, whereas laudanine degraded to compounds **9**, **13**, **18**, and **23**. Analytical data were provided to support the proposed structures for these impurities.

A comprehensive review of the heroin authentic database indicated that the entire set of compounds were prevalent in the SWA and SA categories. For this reason, it would be difficult to differentiate an SWA sample from an SA sample using only these compounds for comparison. To date, other key forensic markers are used to differentiate SWA samples from SA samples.

The MEX samples were rich in stilbene compounds and show distinct differences from the other categories. The SEA samples were for the most part free of theses impurities.

This work has established an entire new series of heroin markers that can be used to help characterize samples. These compounds used in combination with other recognized heroin markers help fortify the final heroin classifications and strengthen the heroin signature program.

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