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CASE REPORT

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Chemical Profiling of Heroin Recovered from the North Korean Merchant Vessel Pong Su

ABSTRACT: Heroin samples, seized from the North Korean merchant vessel Pong Su in Australian waters, were analyzed to determine geographic origin. Duplicate samples were analyzed by the National Measurement Institute's Australian Forensic Drug Laboratory and the United States Drug Enforcement Administration's Special Testing and Research Laboratory. Alkaloid ratios were determined by both liquid chromatography-diode array detection (LC-DAD) and capillary electrophoresis-diode array detection (CE-DAD) techniques. Acid/neutral manufacturing by-products were determined by solvent extraction followed by gas chromatography-mass spectrometry (GC-MS). Solvents, trapped in the heroin particles during manufacture, were detected by both static headspace GC-MS and purge and trap GC-MS. The alkaloid ratios obtained were consistent with heroin of a Southeast Asian (SEA) origin and principal component analysis of the alkaloid results demonstrated the presence of at least four subgroupings within the seizure. The solvent analysis detected diethyl ether and ethyl acetate, solvents typically seen in SEA heroin. However, the acid/neutral analysis revealed compounds not normally seen in heroin of a SEA origin. Furthermore, sterol-like molecules, always detected in the acid/neutral analysis of SEA heroin, were absent from the Pong Su samples. The Pong Su heroin, although similar to SEA heroin, has sufficient differences to classify it as having an unknown origin at the time of this writing.

KEYWORDS: forensic science, chemical profiling, origin classification, heroin, alkaloids

Australia is a large continent with a coastline exceeding 35,000 km, and as a major trading nation it is visited each year by many merchant ships. Such an extensive coastline offers many opportunities for illicit drug importation. In April 2003 a North Korean-flagged merchant vessel passed close to the Australian coastline at Lorne, Victoria. Six packages were placed in a rubber boat for transfer to the shore. On route to shore it is alleged that one package fell overboard and its contents were never recovered. On April 16, 2003, based on intelligence received by Australian Federal Police (AFP) officers, two packages were intercepted by federal agents from the luggage boot of a motor vehicle. On May 7, 2003, the remaining three packages were located by AFP agents buried near the township of Lorne. The foreign merchant vessel, which had been shadowed by a Royal Australian Navy ship, was eventually boarded and the crew and officers arrested (1).

Forensic deconstruction by AFP scientists revealed that the two recovered packages from the luggage boot contained two inner packages, each containing 36 rectangular white blocks of compressed powder, making a total of 144 blocks. Similarly, the three packages buried near Lorne contained two inner packages, each containing 36 rectangular blocks, making a total of 216 blocks. In total, 360 blocks were recovered from the five packages. The average weight per block was determined to be *c*. 350 g. Initial field tests indicated the material was heroin. Each block was individually wrapped with plastic wrapping material stamped with the "Double UO GLOBE" logo. One hundred blocks were randomly selected and 1 g core samples from each of the 100 suspected heroin blocks were sent to the National Measurement Institute's Australian Forensic Drug Laboratory (AFDL) for identification and purity analysis. Because of the nature and size of the seizure, it was decided to carry out chemical profiling on the samples to determine geographical origin. Determination of the major alkaloids, acid/neutral manufacturing by-products, and occluded solvents in heroin has been used by law enforcement agencies to determine geographic origin and drug trafficking routes for many years. A knowledge of the ratios of the alkaloid impurities present in illicit heroin to the total morphine content of the sample allows the chemist to assign origin (2,3), i.e., Southeast Asian (SEA), Southwest Asian (SWA), South American (SA), or Mexican (Mex). This assignment is achieved by comparing these alkaloid ratios with the same ratios obtained on a large number of authentic samples. A knowledge of the occluded solvents provides further evidentiary material for this rough geographic assignment. Solvents used in the processing of opium through to heroin can be indicative of the geographic origin (4). A further aid to the assignment of origin is afforded by the analysis of the acidic and neutral molecules contained within the heroin. During the transformation of opium to heroin, a large number of by-products are formed. The acetylation of morphine to diacetylmorphine involves drastic reaction conditions, and other coextracted alkaloids

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such as thebaine, papaverine, and noscapine undergo chemical reaction. Important among the by-products are various *N*-acetyl derivatives of noscapine, thebaine, morphine, papaverine, and codeine (5). The chromatographic impurity pattern afforded by these acidic and neutral compounds has proven useful in profiling heroin (2,5,6). The method used by the United States DEA's Special Testing and Research Laboratory (STRL) based on work by Allen et al. (5) has been successfully applied to heroin profiling in a number of laboratories. It is achieved by partitioning the neutral and acidic by-products from the basic heroin sample matrix containing the major alkaloids by using an acid/organic solvent mixture.

Most heroin seizures made by AFP officers at the Australian border are of SEA origin and principally involve Burma, Laos, and Thailand (7). Other sources of heroin in the world are Southwest Asia, which today refers mainly to Afghanistan, and South America and Mexico. Analyses of the major alkaloids, acid/neutral manufacturing by-products, and occluded solvents were performed on the Pong Su heroin samples. Data so acquired were added to the Australian Illicit Drug Intelligence Program's database. Duplicate samples were sent to the STRL of the United States Drug Enforcement Administration.

Experimental Section

Reagents and Standards

All reference standards, internal standards, and surrogate standards used in the chemical profiling at AFDL were obtained from the reference collection of the National Measurement Institute. Bis-trimethylsilyltrifluoroacetamide (BSTFA) was obtained from Progen Biosciences (Archerfield, Qld, Australia). Analytical grade methanol was obtained from Malinckrodt Chemicals (Phillipsburg, NJ) and analytical grade dichloromethane and acetonitrile from Merck (Kilsyth, Vic. Australia). Hexylamine, propiophenone, and benzpinacolone were obtained from Aldrich (Castle Hill, NSW, Australia) and were used without further purification.

Major Alkaloid Analysis by Liquid Chromatography at AFDL

The heroin samples were analyzed for the alkaloids noscapine, papaverine, acetylcodeine, codeine, morphine, 6-monoacetylmorphine (6-MAM), 3-monoacetylmorphine (3-MAM), and diacetylmorphine by the method of Lurie and McGuiness (8). Internal standard solutions were prepared by accurately weighing 50 mg of propiophenone into a 100 mL volumetric flask and diluting to volume with mobile phase. Each heroin sample was homogenized by lightly crushing and mixing in a mortar and pestle. Sample (15–20 mg) was accurately weighed into a 10 mL volumetric flask, internal standard (1 mL) added, and the whole diluted to volume with mobile phase and transferred to an injection vial. Solutions of morphine, codeine, noscapine, 6-MAM and 3-MAM (5, 10, 25, 50, and 100 mg/L), acetylcodeine (10, 50, 100, 150, and 200 mg/L), heroin (50, 100, 250, 500, and 1000 mg/L), and papaverine (1, 5, 10, 25, and 50 mg/L) for calibration were prepared from a stock solution made by weighing 10 mg of parent compound into 10 mL volumetric flasks and diluting to volume with mobile phase. Appropriate aliquots and internal standard (1 mL) were diluted accordingly to give the calibrator solutions. Chromatography was performed using an HP-1090 liquid chromatograph (Agilent Technologies, Waldbron, Germany) equipped with a 150 mm × 3.2 mm × 5 μm Alltima C_{18}-LL column. The flow rate was optimized at 0.75 mL/min. The mobile phases were methanol (A) and an amine-phosphate buffer (B). The amine-phosphate buffer was prepared by diluting 30 mL of 2 N NaOH, 11.5 mL of 85% phosphoric acid, and 3.5 mL of hexylamine to 1 L with Milli-Q water and filtering. The gradient profile commenced at 5% A and 95% B and changed linearly to 30% A and 70% B in 20 min, held for 6 min and changed linearly to 80% A and 20% B in 10 min, held for 4 min and then allowed to return to 5% A and 95% B. The injection volume was 20 μL and the detection wavelength was 240 nm.

Major Alkaloid Analysis by Capillary Electrophoresis at STRL

Samples were analyzed for heroin and the major alkaloids using capillary zone electrophoresis with dynamically coated capillaries by the method of Lurie et al. (9). Standards, except for O6-monoacetylmorphine hydrochloride, which was prepared separately, were prepared as a mixture in the injection solvent, which was a 2:8 mixture of methanol and 3.75-mM phosphate buffer (pH 3.2) (9). Each sample was weighed out as a 20-mg equivalent of heroin HCl (based on separate analytical results), placed into a 50-mL volumetric flask (final concentration 0.4 mg/mL), and diluted to volume with injection solvent. After a 15 min sonication, the preparation was filtered and 1 mL was added to a 2-mL glass CE vial and analyzed on an Agilent CE Model #G1600AX using an HPCE standard capillary 50-μm internal diameter (i.d.) and following the method as described by Lurie et al. (9).

Solvent Analysis by Purge and Trap Gas Chromatography-Mass Spectrometry (GC-MS) at AFDL

Occluded solvents were qualitatively determined by purge and trap GC-MS by a method used by the National Measurement Institute's Trace Organics Laboratory (10). Each heroin sample (20 mg) was weighed into a headspace vial, which was then filled with a 2% aqueous solution of sodium sulfate and capped. The vial was submitted for solvent analysis using a Tekmar 3000 Purge & Trap integrated with an Agilent 6890/5973 GC-MS (5). The purge rate was 40 mL/min for 11 min followed by a dry purge for 3 min and desorption at 260°C for 2 min. The transfer line to the GC was held at 100°C. The GC system was fitted with a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-624 column. The oven temperature was programmed as follows: initial temperature 100°C (4 min) and ramped to 120°C at 7°C/min (0 min) and ramped to 220°C at 15°C/min (2 min). The injector was operated in the split mode at 180°C with a split vent flow of 15 mL/min at 140 kPa. The MSD was operated in the electron ionization mode at 70 eV and a scan range of 34–280. The solvent delay was 1.2 min and the transfer line to the MSD was held at 245°C. The method identified the presence or absence of solvents, including diethyl ether, ethyl acetate, acetone, chloroform, dichloromethane, toluene, benzene and the xylenes, and acetonitrile.

Solvent Analysis by Headspace GC-MS at STRL

Occluded solvent analysis for each sample was determined using a static headspace GC-MS method (4). A 40-mg heroin equivalent for each sample was weighed into a headspace vial. Aqueous sodium sulfate (22%) containing five deuterated internal standards was added to each sample and to each of the low, mid, and high calibration standard solutions. Solvent analysis was performed using static headspace GC-MS (Agilent 7964, Agilent 6890/5973 GC-MS) according to the method of Morello and Meyers (4).

Acid/Neutral Analysis by GC-MS at AFDL

Acid/neutral manufacturing by-products were analyzed using a modification of the method used by STRL as described by Allen et al. (5) and Neumann and Gloger (2). Samples were prepared by weighing 30 mg equivalents (based on the total morphine results obtained from the alkaloid analysis) into 10-mL conical tubes and dissolving in 4 mL of light petroleum (b.p. 40–60°C)/dichloromethane mixture (60/40). Internal standards, 2,2,4-trimethylacetophenone (50 mg/L; 100 µL) and d_9-O6,O3,*N*-triacetylnormorphine (50 mg/L; 100 µL) and the surrogate standard *N*-propionylnorlaudanosine (50 mg/L; 100 L) were added to each sample, reagent blank, and QA sample. Sulfuric acid (4 mL, 0.25 M) was then added to each tube and the sample placed on a rock-and-roll mixer for 10 min. The upper organic layer (3 mL) was removed and concentrated just to dryness under a gentle flow of dry nitrogen in a reacti-vial. The residue was immediately dissolved in 250 µL of a mixture of BSTFA/hexane (50/50), capped, and heated at 70°C for 30 min. After cooling 100 µL was transferred to a limited insert GC vial. An Agilent 6890/5973 GC/MSD equipped with a 30 m × 0.25 mm i.d. DB-5MS column with 0.25-µm film thickness was used. The column temperature was programmed from 100°C (1 min) to 240°C at 6°C/min and then to 280°C at 2°C/min and finally to 320°C at 6°C/min. The injection port temperature was 280°C operated in the splitless mode for 0.3 min. The MSD was operated in the selected ion monitoring mode. The compounds monitored and their target ions are given in Table 1.

After data acquisition, peak areas of the analytes of interest (Table 1) were uploaded automatically as CRD files using the Agilent ChemStation software into the database. The peak area of each analyte was summed and each peak area was then expressed as a percentage of the total area. The ratio of the peak area of each analyte to the peak area of the internal standard, and the recovery of the surrogate standard were also determined.

Acid/Neutral Analysis by GC-MS at STRL

Acidic and neutral manufacturing impurities in heroin samples were isolated and analyzed by GC-MS using a method originally done using gas chromatography–flame ionization (2,6). A 45-mg morphine equivalent of each sample was placed into a centrifuge tube and dissolved in 5 mL of a mixture prepared from petroleum ether (b.p. 20–40°C) (540 mL) and methylene chloride (360 mL). Sulfuric acid (2 N, 4 mL), containing 10% sodium sulfate, was added to each sample and vortexed to extract the acid/neutral fraction into the organic phase. Following centrifugation, the organic phase was isolated, concentrated to dryness, and the residue was derivatized with MSTFA. The derivatized extract was analyzed for acidic and neutral manufacturing byproducts on a Polaris-Q GC-MS system (Thermo Finnegan Corporation, Waltham, MA) in the full scan mode (*m/z* 100–575). The initial injector temperature was 85°C (1.5 min hold) and ramped to 295°C (24 min hold) at 180°C/min. Injection mode was splitless. The initial oven temperature was 60°C (6 min hold time), ramped to 200°C at 40°C/min followed by a second ramp to 250°C at 8°C/min and a third ramp to 295°C (11 min hold time) at 1.5°C/min. The carrier gas was helium at a constant velocity of 52.0 cm/s.

Statistical and Chemometric Analysis of Data

The existence of subgroups within the seizure, and its relation to other Australian seizures of SEA heroin, were of interest. The ratios total codeine/total morphine, noscapine/total morphine, and papaverine/total morphine are used by the UN and DEA (11) to assign the general origin of a sample, and graphs of these provide a useful way of identifying groups within a seizure. Some benefit may be gained from a study of the principal components of the data. A principal component is a linear combination of the data that contains the maximum variance, and so should help to distinguish subgroups within the data. A principal components analysis on the raw data of acetylcodeine, heroin, 6-MAM and noscapine for the 100 samples was performed with mean centering but not standardization, using Matlab (v 7.0, Mathworks Inc., Natick, MA) and Excel (Office 2002, Microsoft, Seattle, WA) with the add-in XLStat (v 7.5, Addinsoft).

TABLE 1—*Acidic and neutral compounds monitored by gas chromatography-mass spectrometry (Australian Forensic Drug Laboratory).*

Compound	Target Ions
3,4-Dimethoxy-4,5-epoxyphenanthrene	194(100), 253(65)
Thebaol (O4-TMS)	296(100), 326(74)
Acetylthebaol	254(100, 239(73), 296(35)
4-Acetoxy-3,6-dimethoxy-5-[2(NMA)]ethylphenanthrene	265(100), 252(75), 395(40)
O6,O3,*N*-triacetylnormorphine	209(100), 87(95)
d_9-O6,O3,*N*-triacetylnormorphine (ISTD)	210(100), 87(96)
O6,*N*-diacetylnormorphine	266(100), 281(61), 87(54)
N-acetylnorlaudanosine	234(100), 192(82)
N-propionylnorlaudanosine (ISTD)	192(100), 248(95)
Papaverine	338(100), 324(95), 308(36)
Noscapine	220(100), 205(12)
N-acetylnornoscapine	248(100), 206(77), 191(28)
O6,*N*-diacetylnorcodeine	223(100), 369(52), 87(64)
4-acetoxy-3,6-dimethoxy-8-[2(NMA)]ethylphenanthrene	280(100), 267(30), 395(35)
(*E*)-*N*-acetylanhydronornarceine	382(100), 193(98), 455(16)
(*Z*)-*N*-acetylanhydronornarceine	382(100), 193(98), 455(21)
(1*R*,9*S*)-1-acetoxy-*N*-acetyl-dihydroanhydronornarceine	280(100), 252(42)

Results and Discussion

Alkaloid Profiling

The compositions of the major alkaloids in the 100 seized samples analyzed in the AFDL are summarized in Table 2. The bracketed values are the results obtained by STRL. Table 3 summarizes the ratio of major alkaloids to total morphine obtained from these 100 samples. Again the bracketed figures are the ratios obtained by STRL. The results obtained by the two laboratories agree well. Using either set of ratios, and comparing these ratios with those obtained for heroin samples of known origin ("authentics") all the samples analyzed would be categorized as being of a SEA origin (3). This is noteworthy because although the same heroin blocks were examined by both national laboratories, different core sam-

TABLE 2—*Summary of the composition of the major alkaloids in samples seized from the merchant vessel Pong Su.*

	6-MAM (%)	Diacetylmorphine (%)	Acetyl Codeine (%)	Noscapine (%)
Median	0.3 (0.3)	66.0 (67.2)	16.4 (16.5)	0.3 (0.2)
Minimum	0.2 (0.2)	55.4 (53.7)	8.2 (8.6)	0 (0)
Maximum	0.5 (0.6)	76.0 (78.4)	27.2 (27.4)	0.9 (0.9)

Values in parentheses represent major alkaloid results obtained by United States Drug Enforcement Authority's Special Testing and Research Laboratory.

TABLE 3—*Major alkaloid to total morphine ratios.*

	Codeine/Morphine	6-MAM/Morphine	Noscapine/Morphine
Median	27.1 (24.1)	0.5 (0.5)	0.5 (0.3)
Minimum	12.5 (10.7)	0.3 (0.2)	0 (0)
Maximum	52.1 (49.0)	1.0 (0.9)	2.1 (1.5)

ples from these blocks had to be used for the origin determination in each laboratory.

However, an inspection of the data reveals some interesting results. A large number of samples contained unusually high acetylcodeine content, with 17 samples having acetylcodeine levels between 24.7% and 27.2%. Many samples contained higher levels of noscapine than is typical for SEA heroin. While 18 samples contained no noscapine at all, 15 contained noscapine at levels between 0.6% and 0.9%. Figure 1 shows the noscapine/morphine ratio plotted against the codeine/morphine ratio for all samples. The groupings that appear in the plot of Fig. 1 may be enhanced by inspection of the principal components. PC1, which explains 98% of the variance, and has a positive loading for heroin and negative loading for acetylcodeine, when plotted in ascending order, shows at least four clear groups (Fig. 2).

All heroin samples received by the AFDL during the years 2000–2003 were profiled for geographic origin. It was determined that of these, 1533 samples were of SEA origin. A comparison of acetylcodeine and noscapine levels measured in the heroin samples seized from the Pong Su with the 1533 samples classified as SEA is given in Fig. 3. It is apparent that although the Pong Su samples may be classified as SEA based on their alkaloid ratios, some of them are not typical of heroin traditionally produced in this region. While a number of the Pong Su samples fall in the body of the historical heroin SEA seizures, there is a clear group with high codeine and nonzero noscapine that is not typical of earlier analyses.

The solvent analysis performed at both AFDL and the DEA revealed the presence of diethyl ether and ethyl acetate in each heroin sample seized from the Pong Su. This solvent profile has been recognized by chemists working at the DEA's STRL as being typical of SEA heroin (4). Occluded solvents found in heroin samples are indicative of processes used to prepare heroin. Heroin prepared in Southwest Asia typically contains acetone, while heroin produced in Mexico and South America may contain a variety of solvents including methyl ethyl ketone, methyl isobutyl ketone, acetonitrile, acetic acid, and xylenes. Heroin

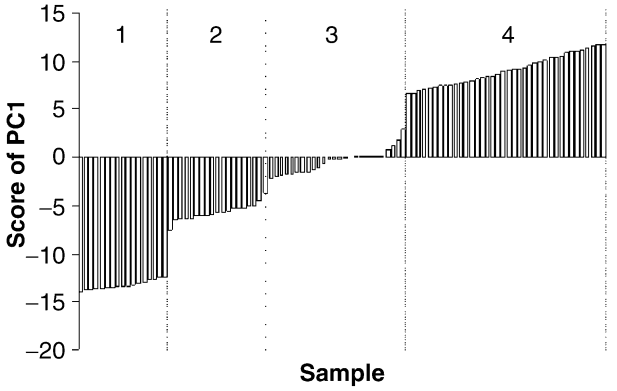

FIG. 2—*Scores of the first principal component of the Pong Su samples in ascending order. Four groups are identified.*

samples examined at AFDL and determined to be from Southeast Asia on the basis of their alkaloid ratios all contained diethyl ether and ethyl acetate.

The analysis of the acidic and neutral components by both laboratories revealed the presence of the following manufacturing by-products in each sample; O6,*N*-diacetylnorcodeine, O6,*N*-diacetylnormorphine, and O3,O6,*N*-triacetylnormorphine. A chromatogram showing the presence of these three compounds in one of the samples seized from the Pong Su is presented in Fig. 4. These three compounds are frequently detected in heroin samples determined, by their alkaloid ratios, to be of SEA origin. They are usually the most abundant *N*-acetylated by-products found in heroin produced in this region. A chromatogram showing the acidic and neutral manufacturing by-products in an authentic SEA heroin sample is given in Fig. 5.

The acid/neutral analysis of each of the seized samples also revealed the presence of 4-acetoxy-3,6-dimethoxy-5-[2(*N*-methylacetamido)ethyl]phenanthrene. This compound is an *N*-acetylated degradation product of the opium alkaloid thebaine. Also detected in each sample was *N*-acetylnornoscapine, which is an *N*-acetylated degradation compound of noscapine. A chromatogram showing these compounds is given in Fig. 4. These manufacturing by-products are not typically seen in heroin of SEA origin but are observed in heroin of SWA origin.

Also of interest was the absence of peaks in the acid/neutral chromatograms that are believed to be due to a group of unidentified sterol-like molecules. The STRL has tentatively identified

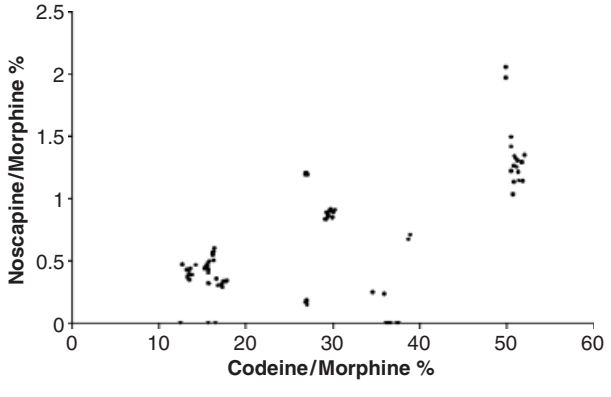

FIG. 1—*Plot of the noscapine to total morphine ratio against the total codeine to total morphine ratio for the 100 samples from the Pong Su seizure.*

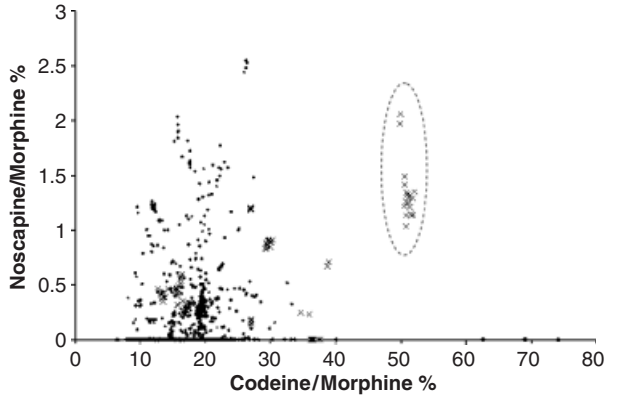

FIG. 3—*Data of Fig. 1 (crosses) with 1533 samples of authentic Southeast Asian heroin seized in Australia during 2002–2003 (closed circles). The group within the dashed oval is that identified as "1" in the PC plot of Fig. 2.*

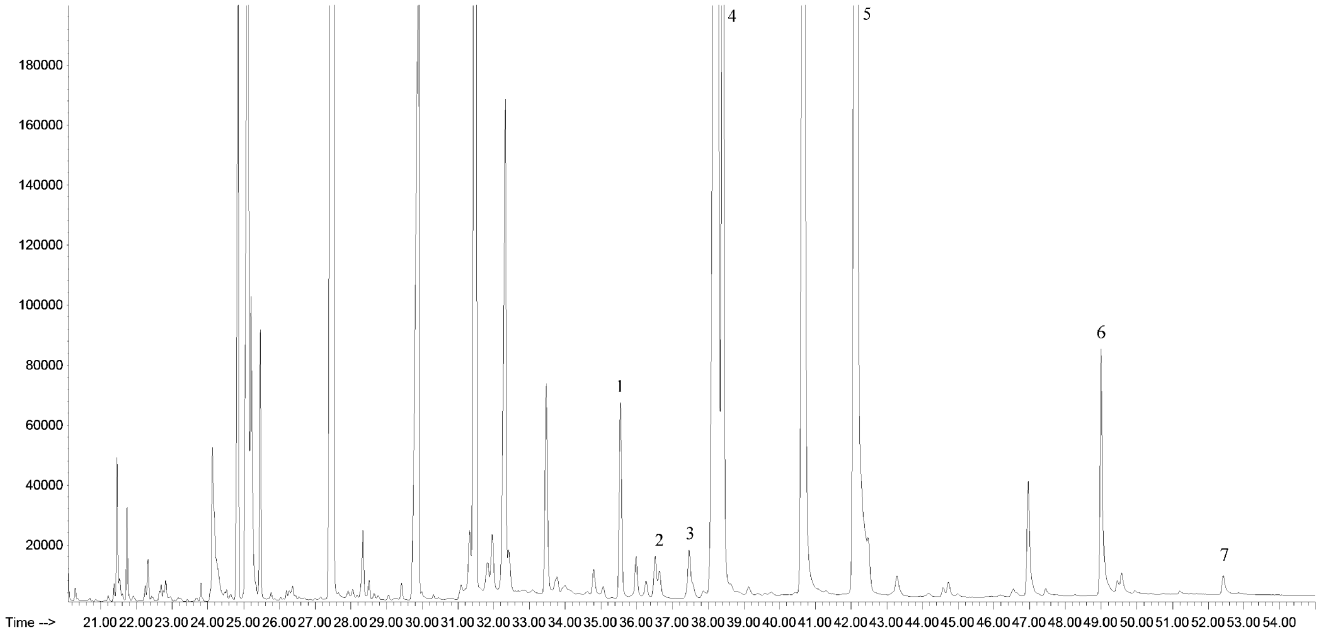

FIG. 4—*Total ion current chromatogram of the acid/neutral extract of a heroin sample seized from the Pong Su. (1) O6,N-diacetylnorcodeine, (2) O6,N-diacetylnormorphine, (3) 4-acetoxy3,6-dimethoxy-5-[2-N-methylacetamido]ethylphenanthrene, (4) O6,O3,N-triacetylnormorphine, (5) noscapine, (6) N-acetylnornoscapine, and (7) 1-acetoxy-N-acetyl-1,9-dihydroanhydronornarceine.*

the presence of these molecules, only observed to date in heroin of known SEA origin, as sterols. While the exact structures of these sterols are as yet unknown, their mass spectral fragmentation patterns are consistent with molecules having the steroid nucleus. The chromatographic peaks of this group of compounds, found in an earlier seizure from Southeast Asia, can be seen in Fig. 5. A mass spectrum of one of these compounds, tentatively identified as a sterol molecule, is shown in Fig. 6. These sterols were not detected by either AFDL or STRL in the samples seized from the Pong Su. Both national laboratories have detected these sterol-like molecules in all authentic SEA heroin samples examined to date. The absence of these compounds in the heroin seized from the Pong Su is noteworthy.

Two possible explanations may be that (1) opium of SWA origin is being transported into Southeast Asia for processing into heroin or (2) a new form of heroin chemically similar to the SEA heroin and processed in a very similar way has been produced. Evidence for the latter suggestion is the observation of an increasing noscapine concentration in SEA heroin in Australia over the last 4 years. One possible explanation for the variation within a single shipment is the theory of central collection, pooling, and distribution points for either the heroin or the opium.

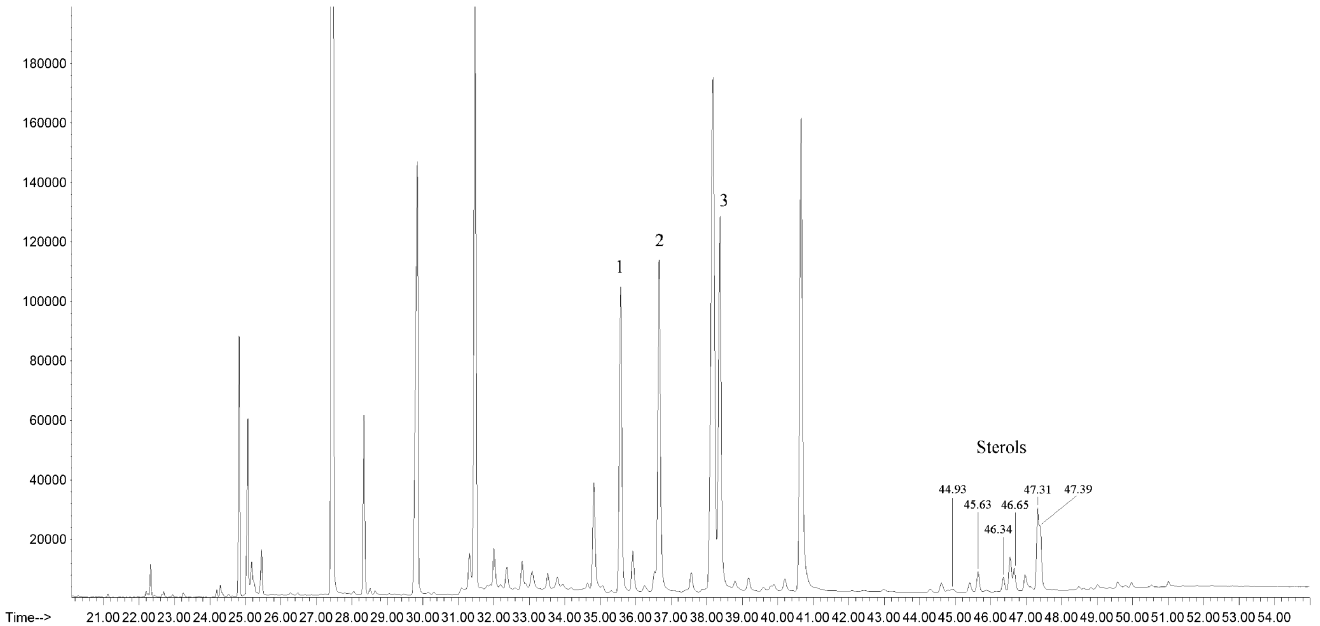

FIG. 5—*Total ion current chromatogram of the acid/neutral extract of a typical SEA heroin sample seized in Australia. (1) O6,N-diacetylnorcodeine, (2) O6,N-diacetylnormorphine, and (3) O6,O3,N-triacetylnormorphine.*

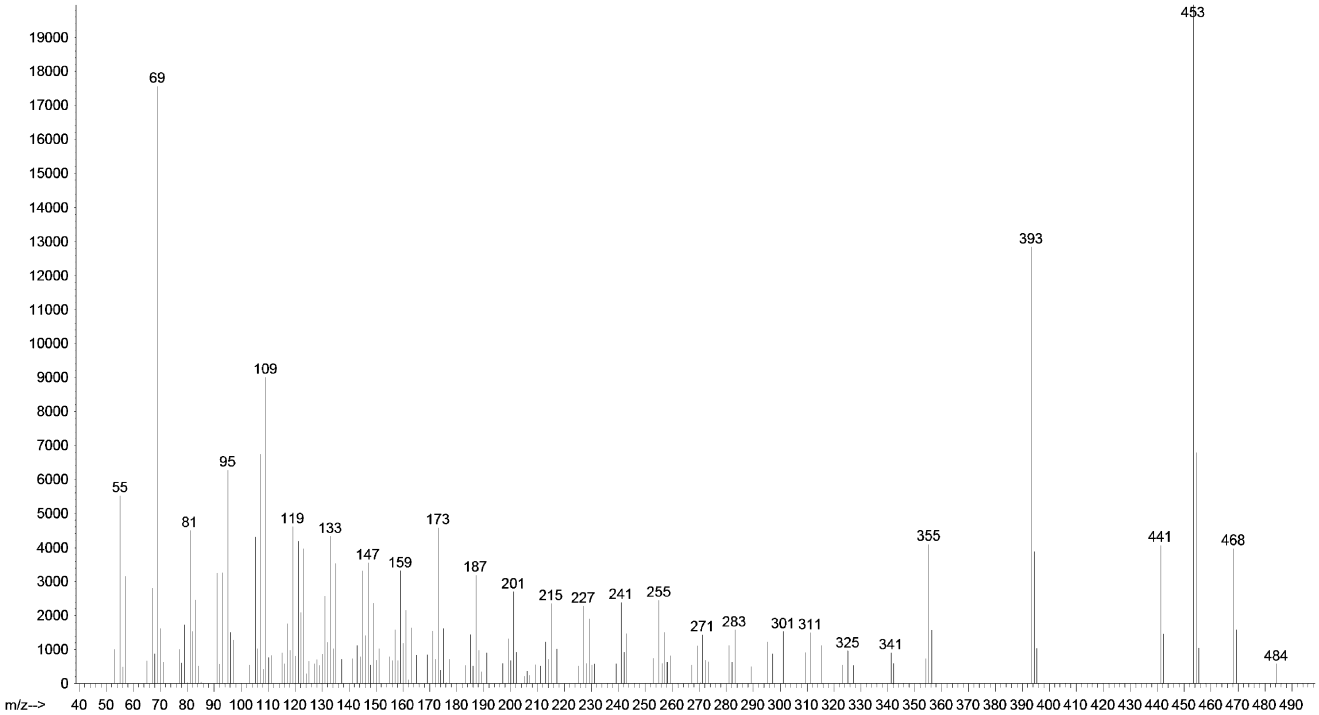

FIG. 6—*Mass spectrum tentatively identified as being due to a member of a group of sterol-like molecules detected only in heroin of a Southeast Asian origin.*

Conclusions

Despite having major alkaloid and occluded solvent profiles consistent with a SEA origin, the heroin seized from the North Korean merchant vessel "Pong Su" was classified as of unknown origin. This decision is based on the presence of compounds in the acid/neutral analysis normally not seen in heroin from this region and the absence of sterols that are normally present in SEA heroin. The presence of acid/neutral manufacturing byproducts common to heroin of SWA origin, in heroin samples with an otherwise SEA profile, is at present inexplicable. Future heroin seizures will be examined for evidence of a similar profile.

Further work, focusing on the carbon stable isotope ratios of authentic heroin samples from Southeast Asia, Southwest Asia, South America and Mexico, and the Pong Su samples, has been carried out at the STRL (12). This work and the alkaloid analyses have demonstrated that the Pong Su samples are different from any heroin previously encountered. However, the geographic origin of this seizure will remain unknown until an authentic heroin sample with a matching profile is obtained.

Acknowledgments

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