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CASE REPORT

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Stable Isotope Analyses of Heroin Seized from the Merchant Vessel Pong Su

ABSTRACT: A new type of heroin HCl seized in Australia was examined by stable isotope analysis. The final origin/process classification of these samples by chromatographic signature profiles of the impurity/manufacturing by-products was previously determined to be "unknown" by two independent national laboratories. Various drug enforcement authorities speculated that the heroin might be from a new region or new illicit process due to the unusual chromatographic impurity profiles that were present. Samples from 20 different kilogram packages were examined for isotopic content to determine if the samples fit isotopic patterns of known origins or if they were unique to any known origins. Authentic specimens from Southeast Asian ($N = 59$), Southwest Asia ($N = 37$), South America ($N = 104$), and Mexico ($N = 21$) we concomitantly examined for comparison purposes. Both continuous flow elemental analysis-isotope ratio mass spectrometry and gas chromatography-isotope ratio mass spectrometry techniques were utilized. Heroin samples were also converted to morphine, without apparent isotopic fractionation, utilizing methanolic HCl for gas chromatography-isotope ratio mass spectrometry. The Pong Su samples were found to be isotopically and isotopically/alkaloidally distinct from the known origin/process classifications of Southwest Asian, Southeast Asian, South American, and Mexican.

KEYWORDS: forensic science, stable isotope ratio mass spectrometry, origin classification, heroin, morphine

Australian authorities seized the North Korean-flagged cargo vessel Pong Su in connection with 50 kg of heroin hydrochloride seized in April 2003. An additional 75 kg was also seized from the off-load site on Australian soil. Routine analysis of the exhibits determined the composition to be heroin HCl and acetylcodeine HCl in varying amounts, where the sum totaled 90–100% (1). It was reported that both Australian and United States authorities suspected it was "highly likely" that North Korea was dealing in illegal drugs (2). The Australian National Measurement Institute's Drug Profiling Laboratory (DPL) and the U.S. Drug Enforcement Administration's (DEA) Special Testing and Research Laboratory joined efforts to determine the possible source of this heroin.

In-depth chromatographic signature profiles of the impurity/manufacturing by-products for 100 samples were conducted by both national laboratories (3). Both laboratories found that the major alkaloids and occluded solvent profiles were consistent with heroin of a Southeast Asian (SEA) origin. However, the acid/neutrals signature profiles (SIG II) were inconsistent with typical heroin of SEA origin. Both laboratories determined that these samples were sufficiently different from typical SEA heroin and classified them as from an unknown origin/process. Intelligence from various sources speculated that the heroin was from a new region or new illicit process.

A subsampling of 20 exhibits was taken for stable isotope determinations. Isotope ratio mass spectrometry (IRMS) has been utilized for several years as an added comparison tool and for origin assignment of heroin samples (4–10). We examined the isotopic content of the heroin, acetylcodeine, and their deacetylated products, morphine and codeine, to determine if the Pong Su samples fit isotopic patterns of known origin (South American, Mexican, Southwest Asian, or Southeast Asian) or if they were unique. Both continuous flow elemental analysis-isotope ratio mass spectrometry (EA-IRMS) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) techniques were utilized for determining the isotope ratios of carbon ($\delta^{13}C$), while EA-IRMS was utilized for determining the isotope ratios of nitrogen ($\delta^{15}N$).

Materials and Methods

Drug Materials and Reagents

Secondary isotopic internal standards of heroin base, morphine HCl, atropine base, codeine base, and cocaine HCl of known carbon and nitrogen isotope abundances were utilized during isotope ratio analyses and were part of an authentic reference collection of this laboratory (DEA). All solvents were distilled in glass products of Burdick and Jackson Labs (Muskegon, MI). *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of reagent-grade quality. Twenty of the 100 individual kilogram Pong Su samples were randomly selected for isotopic analysis after chemical signature analysis had been completed. Additionally, authentic heroin samples (of the DEA laboratory) from Southeast Asia, Southwest Asia, South America, and Mexico were also utilized for comparative purposes.

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Sample Preparation for EA-IRMS

The heroin exhibits (1–2 mg/sample) were combusted directly without further purification. The heroin exhibits were also converted to morphine by the deacetylation procedure as previously described for direct combustion (10).

Sample Preparation for GC-IRMS (Heroin and Morphine)

Approximately 1 mg equivalent of heroin HCl was placed into a 4-mL vial with 1.0 mL of anhydrous $CHCl_3$, capped, and warmed at 55°C for 15 min. After cooling, 25 μL was transferred to two separate autosampler vials, and then evaporated to dryness under a stream of nitrogen. The first vial was derivatized with a mixture of 250-μL ethyl acetate and 250-μL MSTFA for 30 min while capped. The resulting product was suitable for GC-IRMS analysis. The second vial containing heroin was deacetylated by adding 100 μL of 3.5 M methanolic HCl and heated at 75°C for 15 min while capped. The vial was then decapped and residual methanolic HCl was evaporated under a gentle stream of N_2 at 75°C. The resulting morphine sample (deacetylated heroin) was derivatized in the same manner as heroin with ethyl acetate/MSTFA before GC-IRMS.

EA-IRMS and GC-IRMS

EA-IRMS analysis for N_2 and CO_2 was conducted utilizing a Costech model ECS-4010 elemental analyzer coupled with a Thermo Finnigan Delta^{plus} XP IRMS (Bremen, Germany). Samples were combusted to N_2, CO_2, and H_2O over chromium oxide and halogens were trapped in silvered colbaltous oxide. The effluent containing residual nitrous oxides were reduced to N_2 through a copper reduction column and water was removed by an anhydrous magnesium perchlorate trap. The N_2 and CO_2 were separated on a 3-m PQS molecular sieve column before introduction into the mass spectrometer.

GC-IRMS analysis for CO_2 was conducted utilizing an Agilent model 6880 (Palo Alto, CA) gas chromatograph coupled with a Finnigan MAT 252 IRMS. Samples were injected (1 μL) in the splitless mode at an injection port temperature of 250°C. The GC system was fitted with a 30 m × 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μm) (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: level 1, initial temperature, 70°C; initial hold, 1.0 min; temperature program rate, 50°C/min; final temperature, 230°C; final hold, 0.0 min; level 2, temperature program rate, 10°C/min; final temperature, 295°C; final hold, 11.0 min. Helium carrier gas was maintained at a constant linear velocity of 35 cm/s. After the components were separated, the eluent passed through an oxidizing reactor operated at 980°C and then through a reducing reactor set at 640°C. Water was removed via a Nafion tube before introduction of the CO_2 into the mass spectrometer.

Isotopic composition is expressed in δ notation for the isotope ratio (δ, in units of ‰) as

$$\delta = (R_{sample}/R_{standard} - 1) \times 1000$$

where R is the ratio of heavy to light isotopes. The isotope ratios of nitrogen and carbon were determined by comparison with calibrated secondary isotope standards as previously described. Results are reported as $\delta^{15}N$ and $\delta^{13}C$ as ‰ relative to $N_{2\,atm}$ and PDB, respectively. The analytical precision (1σ) for $\delta^{15}N$ and $\delta^{13}C$ measurements were 0.1‰ or better for each system utilized. Assuming parametric statistics, then the 95% confidence interval (2σ) for an average observation should be 0.2‰. Then, when comparing values we assume that $\delta^{15}N$ and $\delta^{13}C$ measurements that differ by 0.3‰ or greater are different from each other.

Results and Discussion

EA-IRMS Analysis

The Pong Su heroin samples were first combusted in their unadulterated form for determination of the stable isotopes of nitrogen and carbon. This method allowed an initial determination of any similarities/dissimilarities within the sample set. The isotope data for nitrogen and carbon, as well as previously determined alkaloid data (3), are illustrated in Table 1. The heroin samples were slightly depleted in ^{15}N (average of –1.5‰) with a relatively tight standard deviation for the data set and possessed average $\delta^{13}C$ values of –32.8‰ of even closer standard deviation. When the $\delta^{15}N$ values for the samples were plotted against purity (Fig. 1), five distinct groupings of the heroin were evident. It should be noted that the $\delta^{15}N$ values represent total nitrogen in the samples and are representative of the combination of heroin and acetylcodeine. Nitrogen isotopic fractionation during illicit heroin processing has been reported (10); these groupings are most likely due to slight differences in the illicit production process, or differences in the acetylcodeine content. The data suggest that the five individual groups of samples were processed in a similar manner (processing) using materials of similar isotopic content (origin).

Morphine was next isolated from the samples by deacetylation of the heroin and examined. Values for $\delta^{15}N$ were significantly more depleted (average = – 4.1‰, standard deviation = 1.25). This result suggested significant fractionation of nitrogen in the morphine isolation procedure for these particular samples. This was apparently due to the acetylcodeine content. Figure 2 illustrates a direct relationship between the acetylcodeine content and apparent fractionation of nitrogen for morphine during the isolation procedure. The relationship was linear with $r^2 = 0.946$. Therefore, the data suggests that the greater the acetylcodeine content, the greater the fractionation of nitrogen for morphine during isolation for EA analysis. Because high acetylcodeine content produced apparent significant fractionation of nitrogen in the purified morphines, we examined the samples via GC-IRMS.

TABLE 1—*Alkaloid and EA-IRMS data derived from 20 Pong Su heroin samples.*

					Heroin*		Morphine*	
	Heroin (%)	Acetylcodeine (%)	Noscapine (%)	Summed (%)	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Average	78.9	18.0	0.3	97.3	– 1.5	– 32.8	– 4.1	– 32.5
Standard deviation (1σ)	8.3	6.8	0.2	1.9	0.51	0.17	1.25	0.27

*Values expressed as ‰ relative to PDB for carbon and $N_{2\,atm}$ for nitrogen.
EA-IRMS, elemental analysis-isotope ratio mass spectrometry.

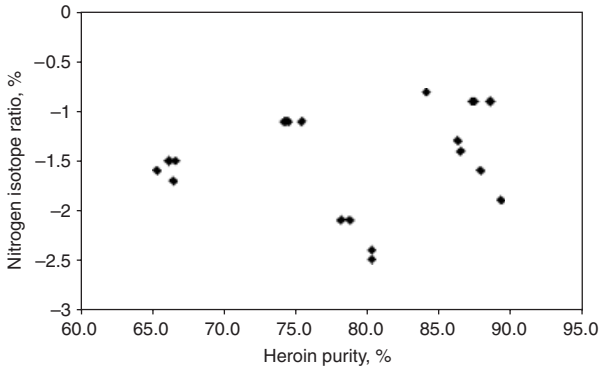

FIG. 1—*Nitrogen isotope ratios[a] (‰) vs. heroin purity of the Pong Su heroin samples. [a]Determined via elemental analysis-isotope ratio mass spectrometry.*

GC-IRMS Analysis

The advantage of GC-IRMS over EA-IRMS is that isotopes can be measured for chromatographically pure compounds. Thus, multiple compounds such as heroin and acetylcodeine or morphine and codeine can be independently measured for their isotope ratios. In this work, only isotopes of carbon were determined. The measured $\delta^{13}C$ values for heroin, acetylcodeine, and the integrated sum of heroin and acetylcodeine are given in Table 2. As seen, acetylcodeine was slightly more depleted in ^{13}C, while the integrated summed $\delta^{13}C$ value (– 32.7‰) was in close agreement with the EA determined value of –32.8‰. These 20 exhibits were isotopically very similar for carbon. The $\delta^{13}C$ values for over 200 authentic heroin samples from Southeast Asia, Southwest Asia, South America, and Mexico were also measured for comparative purposes and are illustrated in Table 3.

We have previously addressed the isotopic enrichment/depletion phenomenon associated with the acetylation of morphine (10). When morphine is acetylated, four more carbons are added, thus changing the original isotopic ratio for carbon. The isotopic composition of the resulting heroin is a combination of the mass balance equation

$$(0.81 \times \delta^{13}C_{\text{morphine}}) + (0.19 \times \delta^{13}C_{\text{acetic anhydride}}) = \delta^{13}C_{\text{heroin theoretical}}$$

where 0.81 and 0.19 are the carbon weight percent values for morphine and acetic anhydride. However, a kinetic fractionation effect (α) during acetylation causes the resulting heroin to have

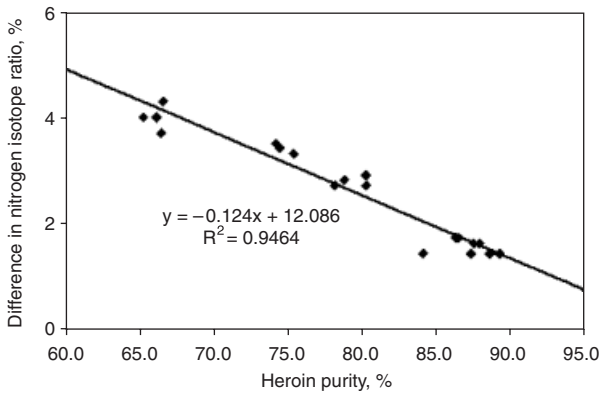

FIG. 2—*Heroin and associated morphine nitrogen isotope differences[a] (‰) versus heroin purity of Pong Su samples. [a]Determined via elemental analysis-isotope ratio mass spectrometry.*

TABLE 2—*δC values (‰) derived from the Pong Su samples (N = 20) via GC-IRMS.*

Compound	Average	Standard Deviation
Heroin	– 32.3	0.10
Acetylcodeine	– 33.6	0.30
Summed heroin+acetylcodeine	– 32.7	0.35
Morphine	– 31.5	0.16
Codeine	– 30.4	0.20
Summed morphine+codeine	– 31.4	0.20

GC-IRMS, gas chromatography-isotope ratio mass spectrometry.

depleted $\delta^{13}C$ values as compared with the morphine starting material (10). Thus, the measured $\delta^{13}C$ values for heroin are a combination of morphine origin and processing. The Pong Su and previously mentioned heroin authentic samples were deacetylated using the described procedure designed to eliminate fractionation effects during sample preparation. The deacetylated samples were subjected to GC-IRMS analysis. The use of MSTFA (derivatizing the two labile protons of morphine) dramatically improves the chromatography of morphine and ensures that all morphine passes from the injection port onto the chromatographic column. Although MSTFA adds six carbons of differing isotopic composition to morphine, a heroin standard synthesized from morphine of known isotopic composition was deacetylated, derivatized, and measured concomitantly. Thus, the MSTFA $\delta^{13}C$ contribution was mathematically subtracted from the derivatized morphine to give actual $\delta^{13}C$ values. The $\delta^{13}C$ values for codeine were analogously determined using a codeine standard of known isotopic composition.

The measured $\delta^{13}C$ values for morphine and codeine derived from the Pong Su samples are given in Table 2. The measured $\delta^{13}C$ values for morphine via GC-IRMS differed from the measured EA-IRMS values by 1.0‰ (Table 1) and may be attributed to fractionation of carbon during the morphine isolation procedure for EA analysis. The $\delta^{13}C$ morphine values for the Pong Su and aforementioned authentic heroin samples from Southeast Asia, Southwest Asia, South America, and Mexico are illustrated for comparative purposes in Table 3. The relative acetylcodeine content to heroin percentages determined from previous works (3, personal communication—DEA) are also given in Table 3. Figure 3 illustrates $\delta^{13}C$ morphine values plotted against $\delta^{13}C$ heroin values for all samples examined using one standard deviation from the mean values. The Pong Su samples were determined to be isotopically distinct from South American, Southeast Asian, Southwest Asian, and Mexican authentics. The *x*-axis is expressed as morphine origin while the *y*-axis is a combination of origin and

TABLE 3—*δC values (‰) via GC-IRMS and alkaloid ratios derived from the Pong Su and authentic heroin samples.*

Region	$\delta^{13}C$ Heroin*	$\delta^{13}C$ Morphine*	Acetylcodeine/ Heroin†
Pong Su unknown (*N* = 20)	– 32.3 (0.10)	– 31.5 (0.16)	24.0 (11.6)
Southeast Asia (*N* = 59)	– 32.7 (0.85)	– 30.4 (0.54)	11.3 (4.43)
Southwest Asia (*N* = 37)	– 32.2 (1.14)	– 29.8 (0.44)	5.0 (2.31)
South America (*N* = 104)	– 32.0 (1.06)	– 31.0 (0.31)	2.4 (0.54)
Mexico (*N* = 21)	– 34.2 (1.35)	– 30.6 (0.46)	5.3 (1.30)

*Average values and (standard deviation).

†Expressed as percent relative to heroin (calculated as acetylcodeine/heroin × 100).

GC-IRMS, gas chromatography-isotope ratio mass spectrometry.

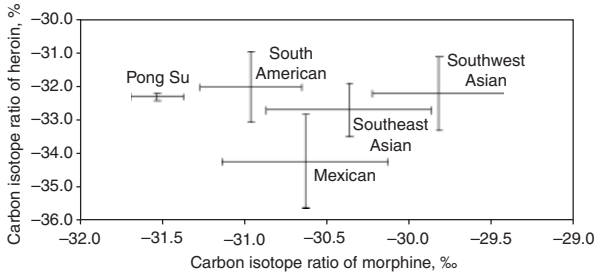

FIG. 3—*Carbon isotope ratios[a] of authentic heroin and associated morphine from the four known source regions (South American, Mexican, Southwest Asian, and Southeast Asian) and the Pong Su samples. Error bars indicate one standard deviation of mean values. [a]Determined via gas chromatography-isotope ratio mass spectrometry.*

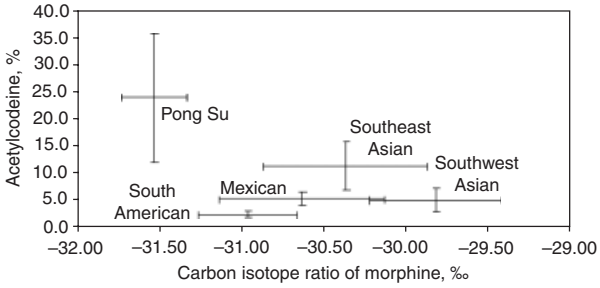

FIG. 4—*Combined alkaloid and isotope data[a]. Acetylcodeine content (relative to heroin) vs. carbon isotope ratio of morphine (‰) from the four known source regions (South American, Mexican, Southwest Asian, and Southeast Asian) and the Pong Su samples. Error bars indicate one standard deviation of mean values. [a]Determined via gas chromatography-isotope ratio mass spectrometry.*

process (acetylation). The Pong Su samples have on average the most depleted $\delta^{13}C$ morphine values, indicating growing conditions different from the four known opium-growing regions. Examples of differing conditions can include a lower altitude or more humid growing environment. Southwest Asian authentic samples have the most enriched $\delta^{13}C$ morphine values and are indicative of the dryer environment of that region. The $\delta^{13}C$ morphine values for the four known growing regions are in general agreement with the previous work of Ehleringer et al. (7). The Pong Su samples are isotopically unique and well separated from known authentic Southeast Asian exhibits.

The combination of isotope and alkaloid data has been used for determining the origins of cocaine (11). In that work, the major coca growing regions of South America were successfully delineated within the same continent, as well as differing growing regions within the same country. The same principle was applied to the samples within this study. Since acetylcodeine is a key discriminating alkaloid for process origin, we applied the relative percentage of acetylcodeine-to-heroin to our morphine isotope data set. It should be noted that the method of opium-to-heroin processing contributes significantly to the acetylcodeine content in heroin samples. Figure 4 illustrates the combination of isotope and alkaloid data. The *x*-axis ($\delta^{13}C$ morphine) remains unchanged from Fig. 3, but the relative overlap within the *y*-axis (percentage of acetylcodeine to heroin) has lessened significantly. In fact, very little overlap of the *y*-axis exists, with the exception of Mexican and Southwest Asian samples. The Pong Su samples are unique and well delineated from the four known origins using the combination of isotope and alkaloid data.

Conclusions

Twenty heroin samples seized in connection with the North Korean-flagged cargo vessel, Pong Su, were examined isotopically and compared with over 200 authentic samples from the four known heroin origins (Southeast Asia, Southwest Asia, South America, and Mexico) and found to be isotopically distinct and unique. The combination of isotope and alkaloid data for these exhibits also illustrated that the Pong Su heroin was unlike any heroin examined previously. The origin of this heroin remains unknown until authentic heroin samples of known origin yielding similar data are acquired.

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