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Isotopic Fractionation of Carbon and Nitrogen During the Illicit Processing of Cocaine and Heroin in South America

ABSTRACT: The forensic application of stable isotope analysis to cocaine and heroin for geolocation of exhibits must take into account the possible enrichment and/or depletion of ^{13}C and ^{15}N during the illicit manufacturing process. Continuous-flow elemental analysis-isotope ratio mass spectrometry was utilized to measure changes in the stable isotope ratios of carbon and nitrogen for both cocaine ($N = 92$) and heroin/morphine ($N = 81$) exhibits derived from illicit manufacturing processes utilized by South American clandestine chemists. In controlled settings in South America, there was no siginficant carbon isotope fractionation during the conversion of cocaine base to cocaine HCl using current illict methodologies. In contrast, nitrogen isotope fractionation for this conversion was 1‰. There was a kinetic carbon isotope ratio fractionation during the acetylation of Colombian morphine to heroin and as a result heroin exhibits will almost always have more negative δ^{13}C values than the original morphine. There was an isotopic fractionation against ^{15}N during the acetylation of morphine base to heroin base, but this effect was not expressed since all of the heroin base was precipitated during the manufacturing process. However, the clandestine process of converting a single batch of heroin base usually involved two consecutive crops of heroin HCl and the latter crop was isotopically depleted as expected from a Rayleigh distillation process. When heroin was deacetylated to morphine, the morphine produced resulted in δ^{13}C values that were indistinguishable from the original morphine. The kinetic carbon isotope fractionation factor for the South American process of morphine acetylation was −1.8‰, allowing calculation of the δ^{13}C values of the acetic anhydride from deacetylated heroin δ^{13}C values.

KEYWORDS: forensic science, stable isotope ratio mass spectrometry, kinetic fractionation, Rayleigh fractionation, cocaine, heroin

The use of isotope ratio mass spectrometry (IRMS) for licit (1,2) and illicit (3–14) drug origin determinations (geosourcing) has increased in popularity for those laboratories equipped to do so. Much of the published work encompasses similar batch comparisons, rather than true geosourcing, due to the lack of sufficient authentic exhibits. To date, only one such stable isotope article on illicit drugs has examined adequate authentic exhibits of absolute known geo-location (latitude and longitude) (4). The utilization of stable isotopes of carbon and nitrogen in drug exhibits is made possible since those atoms become fixed in either the natural (cocaine or morphine), semi-synthetic (heroin or methamphetamine), or the total synthetic (methylenedioxymethamphetamine or methamphetamine) product. Cocaine and morphine are natural products derived from the coca and poppy plants, respectively. Heroin is a semi-synthetic drug produced from acetylation of the natural product, morphine. Examples of illicit drugs, which can be obtained from total synthesis, are methylenedioxymethamphetamine (MDMA), phencyclidine (PCP), and methamphetamine.

Few environmental and biosynthetic fractionation mechanisms are known for cocaine and morphine/heroin (4,5). Incorporation of light or heavy isotopes can follow several pathways in biological systems, which can include cellulose production and alkaloid biosynthesis. Both the coca plant and opium poppy are known to be C_3 plants; their leaf δ^{13}C values are expected to fall in the absolute range of −33‰ to −23‰ (15). The δ^{13}C values for their alkaloids also fall within this range, although they typically tend to be isotopically lighter. The δ^{15}N values for the plants and their natural alkaloids are dependant on both the soil conditions and biosynthetic mechanisms (16–18), with book values ranging between −4‰ and +11‰ for all plants. Known ^{15}N values for cocaine and heroin typically range between +3‰ to −16‰.

The isotope ratio (δ^{15}N and δ^{13}C) values for both illicit cocaine and heroin exhibits are altered by fractionation events during clean up and purification. It should be emphasized that these events are a change in the average isotopic composition in the collection of molecules that constitute the exhibit, and not changes in the atoms of individual molecules. Since illicit cocaine and heroin are not pure compounds but instead mixtures, to some degree the changes in exhibit isotope ratio reflect lesser amounts of other natural products as the materials are purified. Little attention has been paid to the potential isotopic fractionations in going from raw plant materials to these finished alkaloid products. In the course of our work with cocaine and heroin, we have noticed isotopic differences in the free base and HCl ion-pair forms of these drugs which require explanation if isotope ratio analyses are to be used as a forensic tool.

There are two types of isotopic fractionation to be considered: 1) phase change or equilibrium fractionation and 2) kinetic fractionation (19). An example of introducing a phase change fractionation would be manipulation of the salt form for the drug and is usually dependant on the equilibrium of the system. This can include a Rayleigh fractionation, in which the isotope ratio of the finished product is a function of the fraction precipitated from solution. A kinetic fractionation event would be introduced by a reaction, which would either involve atomic exchange or manipulation (addition or deletion of atomic species). An example of this would

be the acetylation of morphine with acetic anhydride. Knowledge and understanding of these isotopic fractionation events is critical to evaluating data obtained from both authentic and non-authentic exhibits, especially when attempting to classify an exhibit by geographic origin. In this work, we normalize any isotope effects from environmental factors by using starting materials (cocaine base and morphine base) of known geolocation.

Materials and Methods

Drug Materials and Reagents

Authentic cocaine base was obtained from Peru. Authentic Colombian morphine was obtained from Colombia. Pharmaceutical-grade cocaine base was a product of Merck Pharmaceutical. Atropine (TCI America) and "Utah cabbage" (5) were utilized as secondary isotopic internal standards during isotope ratio analyses. All other reagents and chemicals were of reagent grade quality, or better, and were used without further purification.

Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS)

Two separate EA-IRMS systems operating in a continuous-flow mode were utilized for this study. For cocaine exhibits, a Carlo Erba model NA-1108 elemental analyzer coupled with a Finnigan MAT Delta-S isotope ratio mass spectrometer was utilized (University of Utah). For the Rayleigh, morphine, and heroin exhibits, analysis was conducted utilizing a Carlo Erba model NA-1110 elemental analyzer coupled with a Finnigan MAT 252 isotope ratio mass spectrometer (Drug Enforcement Administration). Analytical precision for $\delta^{15}N$ and $\delta^{13}C$ measurements were 0.2 per mil or better for each system utilized.

Isotopic composition is expressed in "delta" notation (δ) with the nitrogen or carbon isotope ratio (δ, in units of ‰) as:

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

where R is the ratio of heavy to light isotopes. The isotope ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were determined by comparing to the calibrated secondary isotope standards and international reference standards of N_{2Atm} and Pee Dee Belemnite (PDB). Results are reported as $\delta^{15}N$ and $\delta^{13}C$ as ‰ to N_{2Atm} and PDB, respectively.

A fractionation event has occured whenever the isotope ratio of the substrate and product differ from each other.

Illicit Conversion of Cocaine Base to Cocaine HCl

Ten chemists (6 Colombian, 3 Peruvian and 1 Bolivian) were allowed to process multiple kilograms of authentic Peruvian cocaine base into cocaine HCl in a controlled setting. All clandestine chemists produced a single one-kilogram crop, except for three Colombian chemists who produced slightly smaller initial crops. Representative samples of each cocaine base (starting material) and cocaine HCl (final product) were obtained for IRMS analysis (1–2 mg/analysis).

General Colombian Method: One kilogram of cocaine base was dissolved into a mixture of petroleum ether and methylene chloride (10:1). The solution was then heated to boiling in a hot water bath. A mixture of methyl ethyl ketone and concentrated hydrochloric acid were added to the cocaine base solution. After crystallization was complete, cocaine HCl was captured by gravity filtration, pressed into bricks and microwaved until dry.

General Peruvian Method: One kilogram of cocaine base was dissolved into acetone and heated to boiling. Either a mixture of acetone and concentrated hydrochloric acid, or concentrated hydrochloric acid alone, was added directly to the cocaine base solution. After crystallization was complete, cocaine HCl was captured by gravity filtration, pressed into bricks, and microwaved until dry.

General Bolivian Method: One kilogram of cocaine base was dissolved into diethyl ether and heated to boiling. A mixture of acetone and concentrated hydrochloric acid were added to the cocaine base solution. After crystallization was complete, cocaine HCl was captured by gravity filtration, pressed into bricks, and microwaved until dry.

Illicit Conversion of Morphine Base to Heroin Base and Heroin HCl

Twelve Colombian chemists were allowed to process approximately one kilogram of authentic Colombian morphine into heroin HCl in a controlled setting. Some clandestine chemists produced a single kilogram crop from this morphine, while others produced up to three crops of product. Representative samples of each morphine base (starting material), acetic anhydride (reactant), heroin base (intermediate product), and heroin HCl (final product) were obtained for IRMS analysis (1–2 mg/analysis).

General Colombian Method: Approximately one kilogram of morphine base was refluxed with about twice its weight of acetic anhydride. The reaction was quenched with water and sodium carbonate. The heroin base was then precipitated, filtered, and dried. Approximately 1 kg of heroin base was dissolved in ethyl acetate while heating. A mixture of either acetone or methyl ethyl ketone (MEK) and hydrochloric acid or isopropanolic HCl were added to the heroin base-ethyl acetate solution to precipitate heroin HCl. The heroin HCl precipitation was completed as one or more crops of product. The heroin HCl was then filtered and dried.

Rayleigh Fractionation Experimental Control for the Conversion of Cocaine Base to Cocaine HCl

Pharmaceutical cocaine base (100 g, 0.33 mol) was dissolved into a mixture of 500 mL acetone and 500 mL diethyl ether. While stirring, 100 mL of acetone containing only 3.0 mL of concentrated hydrochloric acid were added rapidly. Cocaine HCl began to precipitate from solution. The precipitation process was allowed to proceed for 10 min, before the precipitate was filtered to collect the cocaine HCl. This process was repeated eight times in succession, with each repetition yielding additional crops of cocaine HCl from the solution. Thus, a total of nine consecutive crops of cocaine HCl were obtained.

Deacetylation of Heroin HCl to Morphine Base

Approximately 50 mg of heroin HCl was placed into a 4 mL vial with 0.25 mL of 5 M NaOH and 0.50 mL of water. The vial was capped and heated at 75°C on a dry bath for 15 min. After cooling, 1.0 mL of water was added. After filtering the solution into a 15-mL centrifuge tube, 2.0 mL of 25% aqueous NH_4Cl was added and the tube scratched to precipitate morphine base. The tube was then centrifuged to provide a plug of morphine base. The supernatant was decanted, and the morphine base washed with 10 ml of distilled water and again centrifuged. This washing procedure was then repeated once more, after which the tube containing morphine base was placed into a vacuum oven overnight to dry at 70°C. The powdered morphine base (purity greater that 98%) was then suitable for EA-IRMS analysis.

TABLE 1—$\delta^{13}C$ and $\delta^{15}N$ values (‰) of cocaine exhibits before and after illicit processing.

Chemist	Cocaine Base		Cocaine HCl		Difference $(\delta - \delta)$	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$
Colombian-1[a,b]	−34.5	−10.3	−34.6	−6.6	−0.1	3.7
Colombian-2[a,b]	−34.5	−10.1	−34.4	−7.7	0.1	2.3
Colombian-3[a,b]	−34.5	−10.2	−34.4	−8.0	0.1	2.2
Colombian-4[a,c]	−34.4	−10.3	−34.4	−9.2	0.0	1.0
Colombian-5[a,c]	−34.4	−10.2	−34.5	−9.3	0.0	0.9
Colombian-6[a,c]	−34.6	−10.0	−34.4	−9.2	0.2	0.8
Peruvian-1[c,d]	−34.8	−10.1	−34.5	−9.1	0.3	1.1
Peruvian-2[c,d]	−34.7	−10.2	−34.5	−9.1	0.2	1.1
Peruvian-3[c,d]	−34.6	−9.8	−34.7	−8.9	−0.1	0.9
Bolivian-1[c,d]	−34.6	−9.8	−34.7	−9.1	−0.1	0.7
Average	−34.6	−10.1	−34.5[b,c]	−9.1[c]	0.1[b,c]	1.0[c]

[a] Average values from five different batches of cocaine base-to-HCl.
[b] One kilogram of cocaine base yielding 800 grams cocaine HCl (non-typical).
[c] One kilogram of cocaine base yielding one kilogram cocaine HCl (typical).
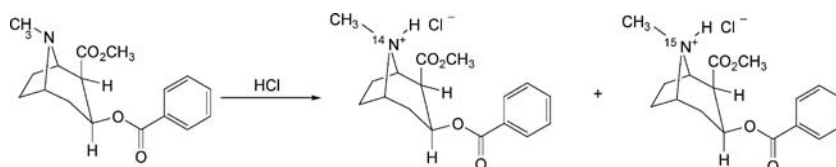[d] Average values from four different batches of cocaine base-to-HCl.

FIG. 1—Formation of cocaine HCl from cocaine base.

Results and Discussion

Cocaine

Each of the clandestine cocaine chemists was provided multiple kilograms of cocaine base to process into cocaine HCl. Each chemist utilized one kilogram of base per batch of cocaine HCl produced. Three of the Colombian chemists (Chemists 1–3) were allowed to utilize a non-typical procedure that is currently obsolete. The non-typical procedure precipitated approximately 800 grams of cocaine HCl by adding 200 mL of concentrated HCl to the process. The remaining unprecipitated cocaine base was extracted from the solutions and would usually be sold as *bazooka*, for smoking purposes. The typical procedure utilized 275 mL of concentrated HCl to produce one kilogram of cocaine HCl. The remaining Colombian, Peruvian, and Bolivian chemists all produced one-kilogram batches of cocaine HCl.

Results from isotopic analyses of the cocaine exhibits associated with the conversion of cocaine base to cocaine HCl are illustrated in Table 1. There were minimal changes in the $\delta^{13}C$ values of cocaine in the clandestine processing, independent of the conversion method utilized. In fact, when all 46 individual batches of cocaine are examined for $\delta^{13}C$, the difference between the base and HCl forms averaged less than 0.1‰. This is not surprising since all of the carbons on the cocaine molecule are essentially non-reactive and are not exchanged during the base-to-HCl process. Even if one of the cocaine carbons was somewhat susceptible to influence by equilibrium exchange effects, sixteen other carbons in the molecule would retain their isotopic integrity and physical properties, thus minimizing a phase change or equilibrium fractionation. The measured $\delta^{13}C$ values (‰) for cocaine are essentially a "mass average" of all carbons throughout the cocaine molecule.

An apparent fractionation against ^{15}N in cocaine occurred during the clandestine base-to-HCl conversion and this is reflected in the differences in $\delta^{15}N$ values of the base and HCl forms of cocaine (Table 1). The nitrogen atom is reactive in this case, as is illustrated in Fig. 1. This apparent fractionation is expected, since the lone pair of electrons on the nitrogen are involved in acquiring the ion-pair of hydrochloric acid. The Colombian chemists (1–3) that produced only 800 grams of cocaine HCl obtained the greatest ^{15}N enrichment in their processing, with average ^{15}N enrichment values ranging from 2.2‰ to 3.7‰. This equilibrium exchange fractionation apparently exhibits a Rayleigh fractionation in which the isotope ratio of the product is dependent on what fraction of the cocaine has been precipitated. Isotopic mass balance is maintained because the unprecipitated cocaine is ^{15}N depleted. Although we were not able to measure the isotopic ratios of the unprecipitated cocaines, their $\delta^{15}N$ values (‰), due to mass balance, would have to be more negative than the precipitated products.

To examine this possibility further, we conducted an experiment in which successive fractions of cocaine were precipitated from solution. Data from this experiment in Table 2 illustrate that the fractionation of nitrogen is approximately two orders of magnitude greater than that for carbon. Figure 2 shows a plot of the isotope ratios of the precipitated cocaine as a function of the total fraction that has been precipitated. These data verify a Rayleigh fractionation. The remaining clandestine chemists obtained one kilogram of product, regardless of illicit methodology, all having similar fractionations of ^{15}N. These exhibits were also ^{15}N enriched, but to a lesser extent, with values ranging from 0.7‰ to 1.1‰. The results also follow the Rayleigh fractionation curve. Although these chemists obtained 1 kg of product from one kilogram of cocaine base, it was approximately an 88% actual yield. The ^{15}N enrichment equilibrium is therefore not as prominent for the higher yielding chemists due to this effect. In theory, if a clandestine chemist had obtained 100% of the theoretical yield, there would be no measurable

TABLE 2—$\delta^{13}C$ and $\delta^{15}N$ values (‰) of Rayleigh fractionation control experiment for the conversion of cocaine base to cocaine HCl.

Chemist	Cocaine Base[a]		Cocaine HCl		Σ % Recovery of Cocaine HCl	% Remaining Cocaine Base
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$		
Crop-0	−32.2	−9.3	NS	NS	0.0	100.0
Crop-1	−32.2	−10.8	−31.9	3.9	10.2	89.8
Crop-2	−32.3	−12.7	−32.0	1.9	20.8	79.2
Crop-3	−32.3	−14.7	−32.0	−0.4	31.9	68.1
Crop-4	−32.3	−17.3	−32.1	−2.9	42.9	57.1
Crop-5	−32.4	−20.3	−32.2	−5.7	53.6	46.4
Crop-6	−32.5	−24.4	−32.2	−9.9	64.2	35.8
Crop-7	−32.6	−29.7	−32.3	−15.7	74.9	25.1
Crop-8	−32.6	−35.6	−32.5	−24.8	85.4	14.6
Crop-9	NS	NS	−32.7	−36.6	90.2	9.8

[a] Crop-0 = cocaine base before any conversion to cocaine HCl. Remaining cocaine base values are from the filtrates of the corresponding precipitated cocaine HCl crops.
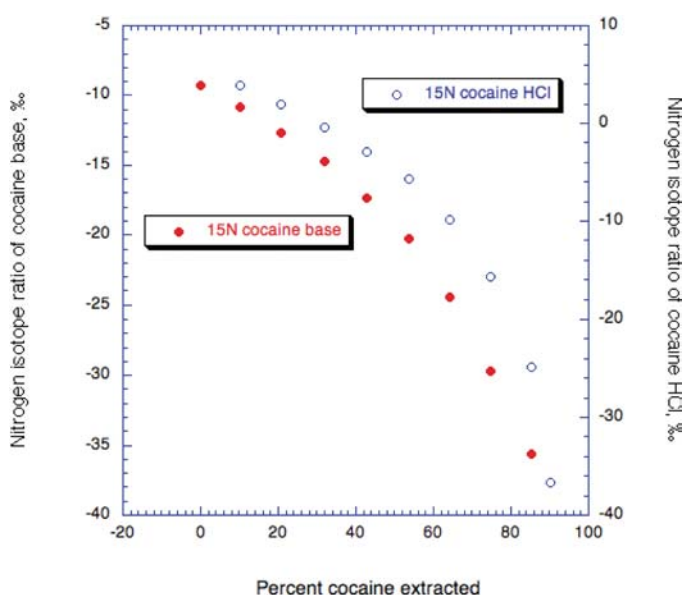
NS = no sample recovered.

FIG. 2—^{15}N isotopic ratios of precipitated cocaine as a function of the total fraction that has been precipitated.

fractionation of nitrogen or carbon. Based on the experimental results, it appears that cocaine containing ^{15}N has a slightly greater affinity to form the HCl ion-pair and precipitate from solution than its ^{14}N species. Overall, the average fractionation of ^{15}N was found to be 1.0‰ for typical (current) processing methodologies throughout South America for the conversion of cocaine base into cocaine HCl.

Acetylation of Morphine Base to Heroin Base

Each of the 12 clandestine chemists produced (precipitated) one major crop of heroin base from the acetylation of morphine. Ten chemists precipitated a second minor crop consisting of at least an order of magnitude less quantity heroin base, while seven chemists managed to precipitate a third crop containing even less material. The $\delta^{13}C$ and $\delta^{15}N$ values (‰) of morphine base and heroin base exhibits associated with these precipitations are illustrated in Table 3. Overall, the fractionation of ^{15}N from morphine to major crops of heroin base is minimal, but considerable in the minor final resid-

ual crops. The ^{15}N content for the first and second crops of heroin base were slightly depleted relative to the morphine base (average of −0.5‰). Chemists that precipitated a minor third crop obtained heroin with significant ^{15}N depletion (average of −7.3‰). These results are analogous to the cocaine data in which a Rayleigh fractionation has occurred in the residual precipitation of heroin in the acetylation process. The ^{15}N heaviest molecules are precipitated first. Isotopic mass balance in this equilibrium system illustrates that ^{15}N depleted heroin base has less affinity to precipitate from an aqueous alkaline solution.

A slight variance was observed in the $\delta^{13}C$ values for the heroin base exhibits as compared to the $\delta^{13}C$ values for the morphine starting material, although the acetic anhydride utilized had a measured $\delta^{13}C$ value of −23.4‰ (Table 3). There is considerable potential for isotopic enrichment/depletion for the produced heroin since four carbons are added to morphine from the acetylation. In theory, enrichment of ^{13}C in heroin would be expected since the acetic anhydride contained a greater ratio of ^{13}C than the morphine. However, this is not the case, as is seen in Tables 3 and 4. The theoretical mass balance equation for this reaction is:

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

$$= \delta^{13}C_{heroin\ theoretical}$$

where 0.81 and 0.19 are the carbon weight percent values for morphine and acetic anhydride, respectively. When using the measured morphine and acetic anhydride values for chemist #6 in this equation, the expected theoretical value for $\delta^{13}C$ is −30.5‰, which differs from the measured value of −31.7‰. The difference was attributed to kinetic fractionation during the acetylation reaction. When the mass balance formula is applied to all chemists, the average kinetic fractionation factor (α) is approximately −1.5‰, with a range of −1.1‰ to −2.0‰ for heroin base. Besacier and co-workers [3] reported a similar fractionation event of −3.3‰, but on a milligram-scale acetylation. We repeated the Besacier work on a one-gram scale (ca. 1000 fold greater) and also obtained the same factor of −3.3‰.

Conversion of Heroin Base to Heroin HCl

Four chemists produced (precipitated) only a single crop of heroin HCl from heroin base, while eight chemists produced two distinct crops. The chemists utilized either concentrated HCl or

TABLE 3—$\delta^{13}C$ and $\delta^{15}N$ values (‰) of morphine base and heroin base.

Chemist	Morphine Base[a] Starting Material		Heroin Base[b,c] 1st Precipitate		Heroin Base[d] 2nd Precipitate		Heroin Base[d] 3rd Precipitate		Heroin Base Wt. % Avg.	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Colombian-1	−32.1	0.8	−32.4	0.3	−31.8	0.6	NS	NS	−32.4	0.3
Colombian-2	−32.0	0.9	−31.6	0.4	NS	NS	NS	NS	−31.6	0.4
Colombian-3	−32.2	0.6	−31.9	0.0	−32.2	0.6	−32.1	−12.2	−31.9	−0.3
Colombian-4	−32.3	1.5	−31.8	1.4	−32.4	−3.0	−32.2	−3.0	−31.8	1.2
Colombian-5	−32.1	0.9	−32.4	0.7	−32.1	−1.6	NS	NS	−32.4	0.5
Colombian-6	−32.2	1.1	−31.7	0.7	NS	NS	NS	NS	−31.7	0.7
Colombian-7	−32.2	1.2	−31.6	0.6	−31.8	1.9	−32.2	−2.2	−31.6	1.4
Colombian-8	−32.3	1.1	−32.0	0.7	−31.9	2.9	−32.4	−7.9	−32.0	0.0
Colombian-9	−31.9	1.2	−31.9	0.8	−27.5	1.1	−32.5	−10.3	−31.9	0.1
Colombian-10	−31.9	1.0	−32.1	0.9	−30.7	3.5	−32.3	−8.2	−32.1	0.3
Colombian-11	−32.4	2.0	−31.8	−0.2	−31.8	−0.2	NS	NS	−31.8	−0.2
Colombian-12	−32.2	1.4	−32.4	1.1	−29.9	1.6	NS	NS	−32.4	1.1
Average	−32.2	1.1	−32.0	0.6	−31.2	0.6	−32.3	−7.3	−32.0	0.4

[a] Weight average values from combined morphine starting materials (N = 3 or 4).
[b] Precipitated heroin base product used for conversion to heroin HCl.
[c] Acetic anhydride with $\delta^{13}C = -23.4$‰ utilized for the acetylation of morphine.
[d] Not utilized for conversion to heroin HCl.
NS = No sample obtained by clandestine chemist.

TABLE 4—$\delta^{13}C$ and $\delta^{15}N$ values (‰) of morphine base, heroin base, and heroin HCl.

Chemist	Morphine Base Starting Material		Heroin Base[a] Intermediate		Heroin HCl-1 1st Precipitate		Heroin HCl-2 2nd Precipitate		Heroin HCl wt. % avg.	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Colombian-1[b]	−32.1	0.8	−32.4	0.3	−33.0	0.4	NS	NS	−33.0	0.4
Colombian-2[c]	−32.0	0.9	−31.6	0.4	−31.8	1.4	−31.7	−7.7	−31.8	1.3
Colombian-3[b]	−32.2	0.6	−31.9	0.0	−32.3	4.3	−32.2	−4.9	−32.3	1.8
Colombian-4[c]	−32.3	1.5	−31.8	1.4	−32.3	2.2	−32.3	−2.6	−32.3	1.6
Colombian-5[c]	−32.1	0.9	−32.4	0.7	−32.3	0.9	NS	NS	−32.3	0.9
Colombian-6[b,d]	−32.2	1.1	−31.7	0.7	−32.3	1.7	−32.0	1.9	−32.1	1.8
Colombian-7[b]	−32.2	1.2	−31.6	0.6	−32.2	2.5	NS	NS	−32.2	2.5
Colombian-8[b]	−32.3	1.1	−32.0	0.7	−32.3	4.8	−32.7	−4.7	−32.5	1.2
Colombian-9[b]	−31.9	1.2	−31.9	0.8	−32.4	1.6	−32.2	−0.4	−32.4	1.6
Colombian-10[c]	−31.9	1.0	−32.1	0.9	−32.2	1.6	−32.2	−5.9	−32.2	1.6
Colombian-11[b]	−32.4	2.0	−31.8	−0.2	−32.3	0.4	−32.6	−3.2	−32.3	0.4
Colombian-12[b]	−32.2	1.4	−32.4	1.1	−32.7	0.6	NS	NS	−32.7	0.6
Average	−32.2	1.1	−32.0	0.6	−32.3	1.9	−32.2	−3.4	−32.3	1.3

[a] Utilized to produce heroin HCl.
[b] Used isopropanolic HCl for conversion of heroin base to heroin HCl.
[c] Used concentrated HCl for conversion of heroin base to heroin HCl.
[d] Washed both heroin HCl crops with fresh MEK.
NS = No sample obtained by clandestine chemist.

isopropanolic HCl for the conversions. Results from isotopic analysis of the heroin HCl exhibits are illustrated in Tables 4 and 5. There are no kinetic isotope fractionation effects for carbon in this situation. Yet, overall there has been a slight ^{13}C depletion in both heroin HCl crops with average $\Delta\delta^{13}C$ values of −0.3‰ and −0.4‰, respectively. Chemist #5 (crop-1) and chemist #11 (crop-2) were exceptions, having a slight enrichment in ^{13}C (0.1‰ and 0.2‰, respectively), which is not statistically significant due to the precision limit of 0.2‰). The distribution of ^{13}C-depleted heroin HCl exhibits was not found to be related to the use of concentrated versus isopropanolic HCl. It is unclear whether the acetate carbons have had any influence on ^{13}C depletion during the HCl conversion process. Interestingly, over half of the heroin HCl crops (N = 13) gave nearly identical $\delta^{13}C$ values (−32.3‰ ± 0.1‰), even though each was a separate batch and usually produced by a different chemist (N = 9). It is clear from the data in Table 4 that $\delta^{13}C$ values for heroin cannot

be utilized for sample-to-sample comparisons (same batch), except to rule out possible relationships. However, minimal fractionation of carbon was observed and may be a useful data point for origin classifications or determining the source of acetic anhydride.

Fractionation of ^{15}N was clearly evident at this stage of the clandestine process (Table 5). With the exception of chemist #12, all preliminary heroin HCl crops became enriched in ^{15}N (0.1‰ to 4.3‰). All secondary crops became depleted except for chemist #6. The average equilibrium fractionation ($\Delta\delta^{15}N$) was 1.2‰ for the first crop of heroin HCl. These data are in close agreement with the Rayleigh fractionation noted during the conversion of cocaine base to cocaine HCl ($\Delta\delta^{15}N = 1.0$‰), which is expected since heroin is also a cyclic tertiary amine. The average equilibrium fractionation ($\Delta\delta^{15}N$) was −4.0‰ for the second crop of heroin HCl. Therefore, heroin containing ^{15}N has a greater affinity to form the HCl ion-pair than its ^{14}N species, as was also found for cocaine. Not surprisingly,

TABLE 5—$\delta^{13}C$ and $\delta^{15}N$ differences (‰) from conversion of morphine base to heroin base and heroin HCl.

Chemist	Morphine Base-To-Heroin Base[a]		Heroin Base-To-Heroin HCl Crop 1		Heroin Base-To-Heroin HCl Crop 2		Heroin Base-To-Heroin HCl wt. % Avg.	
	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$
Colombian-1	−0.3	−0.5	−0.6	0.1	NS	NS	−0.6	0.1
Colombian-2	0.4	−0.5	−0.2	1.0	−0.1	−8.1	−0.2	0.9
Colombian-3	0.3	−0.6	−0.4	4.3	−0.3	−4.9	−0.4	1.8
Colombian-4	0.5	−0.1	−0.5	0.8	−0.5	−4.0	−0.5	0.2
Colombian-5	−0.3	−0.2	0.1	0.2	NS	NS	0.1	0.2
Colombian-6	0.5	−0.4	−0.6	1.0	−0.3	1.2	−0.4	1.1
Colombian-7	0.6	−0.6	−0.6	1.9	NS	NS	−0.6	1.9
Colombian-8	0.3	−0.4	−0.3	4.1	−0.7	−5.4	−0.5	0.5
Colombian-9	0.0	−0.4	−0.5	0.8	−0.3	−1.2	−0.5	0.8
Colombian-10	−0.2	−0.1	−0.1	0.7	−0.1	−6.8	−0.1	0.7
Colombian-11	0.6	−2.2	−0.5	0.6	0.2	−3.0	−0.5	0.6
Colombian-12	−0.2	−0.3	−0.3	−0.5	NS	NS	−0.3	−0.5
Average	0.2	−0.5	−0.4	1.2	−0.3	−4.0	−0.4	0.7

[a] Utilized to produce heroin HCl.
NS = No sample obtained by clandestine chemist.

chemists that produced two crops of heroin HCl obtained primary products with $\delta^{15}N$ values of 0.4‰ to 4.8‰. Except for chemist #6, their secondary crops (second precipitation) were depleted, with $\delta^{15}N$ values of −0.4‰ to −7.7‰ and in agreement with mass balance. Chemist #6 was the exception to this rule; most probably due to the fact that he was the only chemist that washed his heroin HCl products with fresh MEK. In this case it appears that the ^{14}N ion pair has preferential solubility over the ^{15}N ion pair in MEK.

Dramatic differences are observed for $\delta^{15}N$ in two-crop precipitations. Chemists that produced approximately two equal crops by weight obtained products with almost opposite $\delta^{15}N$ values. For example, Chemist # 8 (Table 4) produced two distinct crops of heroin HCl with $\delta^{15}N$ values of 4.8‰ and −4.7‰ ($\Delta\delta^{15}N = 9.5$). Chemists that produced a minor second crop obtained products significantly depleted in ^{15}N, while their preliminary crop retained ^{15}N values more in line with the heroin base (starting material). An example of this phenomenon is chemist #2, where $\delta^{15}N$ values for heroin base, heroin HCl crop-1, and heroin HCl crop-2 were 0.4‰, 1.4‰ and −7.7‰, respectively.

Due to the wide variation in $\delta^{15}N$ values that could be produced for Colombian heroin HCl (4.8‰ to −7.7‰) in the different crops, it is unclear at this point if isotopic ratios of nitrogen would be useful in an origin determination. The results of fractionation against ^{15}N in heroin were influenced by some clandestine Colombian heroin processors and could significantly impact the $\delta^{15}N$ values of heroin HCl obtained for the region.

Deacetylation of Heroin HCl Exhibits

Ten different Colombian morphine exhibits were first subjected to the deacetylation procedure as outlined in the experimental section to determine if the precipitation step would fractionate carbon. Ehleringer and coworkers (5) have previously shown that $\delta^{15}N$ values for morphine are retained from deacetylated heroin. Therefore, our focus was toward the $\delta^{13}C$ values for deacetylated heroin. Since the original $\delta^{15}N$ and $\delta^{13}C$ values of the morphine were known, and the fact that morphine is soluble in aqueous NaOH, heroin was not used. Minimal fractionation of carbon was observed with an average $\Delta\delta^{13}C$ value of 0.1‰. Therefore, as seen in Table 6, the $\delta^{13}C$ values for the deacetylated products will be in close agreement with the original morphine base starting materials.

The $\delta^{13}C$ values for the deacetylated exhibits are illustrated in Table 6 and are compared to their corresponding heroin exhibits.

TABLE 6—$\delta^{13}C$ differences (‰) from original morphine to converted morphine (from heroin).

Chemist	Original Morphine Base	Morphine From Heroin HCl Crop 1		Morphine From Heroin HCl Crop 2	
	$\delta^{13}C$	$\delta^{13}C$	$\Delta\delta^{13}C$	$\delta^{13}C$	$\Delta\delta^{13}C$
Colombian-1	−32.1	−32.1	0.0	NS	NS
Colombian-2	−32.0	−31.7	0.3	−32.0	0.0
Colombian-3	−32.2	−32.2	0.0	−32.2	0.0
Colombian-4	−32.3	−32.3	0.0	−32.4	−0.1
Colombian-5	−32.1	−32.0	0.1	NS	NS
Colombian-6	−32.2	−32.0	0.2	−32.0	0.2
Colombian-7	−32.2	−31.8	0.4	NS	NS
Colombian-8	−32.3	−32.1	0.2	−32.2	0.1
Colombian-9	−31.9	−31.7	0.2	−31.7	0.2
Colombian-10	−31.9	−31.7	0.2	−31.8	0.1
Colombian-11	−32.4	−32.6	−0.2	−32.6	−0.2
Colombian-12	−32.2	−32.4	−0.2	NS	NS
Average	−32.2	−32.1	0.1	−32.1	0.0

NS = No sample obtained by clandestine chemist.

The $\Delta\delta^{13}C$ values for those exhibits are also illustrated in Table 6. These data illustrate the differences between the original morphine and the morphine obtained from deacetylation of the corresponding heroin. Little variation in $\delta^{13}C$ is observed for the corresponding morphines. As seen, average $\Delta\delta^{13}C$ values only vary by 0.1‰, regardless of heroin crop, and have relatively tight standard deviations. In this case, it is clear that when heroin is deacetylated, the morphine produced will give $\delta^{13}C$ values virtually identical to the original morphine starting material. This is fortuitous for two reasons. First, $\delta^{13}C$ values of deacetylated heroin, regardless of the acetylating reagent's $\delta^{13}C$ value, will give evidence to the original morphine values. Preliminary works have shown the potential for $\delta^{13}C$ morphine values being indicative of certain geographical regions. Secondly, strategic intelligence can be obtained by calculating the approximate $\delta^{13}C$ values of the acetic anhydride.

The kinetic isotope effect (α) for the Colombian acetylation process can now be determined using the average $\delta^{13}C$ values for acetic anhydride, heroin HCl and the purified morphine obtained from deacetylation in this work. The kinetic isotope effect is due to the extra neutron on the ^{13}C carbonyl in acetic anhydride. The neutron gives a slightly greater shielding effect, making a stronger covalent

bond between ^{13}C and the anhydride oxygen. Therefore, any ^{12}C carbonyl will break slightly preferentially from the anhydride oxygen and add to morphine. Using the mass balance equation, α of $-1.8‰$ was obtained. We can reconstruct the approximate δ^{13}C values for acetic anhydride that were used to produce illicit heroin HCl from morphine using the following equation:

$$\left(\frac{(\delta^{13}C_{\text{heroin}} + 1.8) - (\delta^{13}C_{\text{morphine}})}{0.19} \right) = \delta^{13}C_{\text{acetic anhydride}}$$

Using the above formula with the average values obtained for heroin HCl and its deacetylated product (morphine), the calculated acetic anhydride value for δ^{13}C is $-23.7‰$, which differs from the actual measured value of $-23.4‰$ by only $-0.3‰$. It is apparent from the one kilogram scale (10^3 to 10^6 fold increase) used in clandestine manufacture, that the kinetic isotope effect is somewhat diminished ($-1.8‰$ vs. $-3.3‰$) when compared to the controlled experiments conducted by ourselves and Besacier. This equation can only be utilized for Colombian heroin (reflux, closed system), because the Southeast Asian, Southwest Asian, and Mexican acetylation reactions are known to be different. Those methodologies must be independantly investigated for their own kinetic effects.

Conclusions

There is minimal carbon isotope fractionation in illicit cocaine processing (base-to-HCl) and values were not affected by different processing methodologies. Fractionation of ^{15}N in illicit cocaine processing was detectable when not all of the cocaine was precipitated. An average $\Delta\delta^{15}$N value of $1.0‰$ was determined for conversion of cocaine base to cocaine HCl using current illicit methodologies.

The isotopic fractionation against ^{15}N during the acetylation of morphine base to "major crops" of heroin base was minimal. However, considerable fractionation against ^{15}N was detectable in the residual minor crops of heroin base. A kinetic fractionation event was observed for the Colombian method of acetylation of morphine. Due to this effect, heroin exhibits will almost always have more negative δ^{13}C values than the original morphine. Fractionation against ^{15}N was significant during the clandestine process of converting heroin base to heroin HCl, especially for chemists that produced two major crops of heroin HCl. The Colombian process for producing heroin can significantly alter the natural isotopic abundances of nitrogen. However, when heroin is deacetylated to morphine, the produced morphine gives virtually identical δ^{13}C values to the original morphine. Furthermore, we can now calculate the approximate δ^{13}C values for the acetic anhydride which was utilized for the morphine acetylation.

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