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Geo-location of heroin and cocaine by stable isotope ratios

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Abstract

Analyses of the carbon and nitrogen stable isotope ratios in heroin and cocaine samples obtained from different geographic regions indicated stable isotope ratio combinations that were strongly correlated with geographic location. Further analyses of the isotope ratios of morphine derived from the deacetylation of heroin exhibited more pronounced isotopic differences among regions, increasing its potential as a tool for geo-location and for sample-to-sample comparison.

Keywords: Forensic science; Heroin; Morphine; Cocaine; Carbon isotope ratio; Nitrogen isotope ratio; Geographic origin

1. Introduction

There is considerable international interest in the identification of the geographic

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origin of illicit drugs, particularly those obtained from natural sources. Thus far, for those drugs obtained from natural sources there have been some limited successes in reliably identifying the geographic origin. Current approaches most often rely on analytical methods based upon elemental or chromatographic analyses, but all of these methods suffer from the same limitation in that they do not explicitly establish geographic source, rather they primarily determine methods utilized in the isolation and preparation of the drug [1–8].

Isotope ratio analyses at natural abundance levels have proven useful in many environmental sourcing studies, spanning such areas as tracing animal migration routes [9–11], determining the geographic origins of linen [12], and sourcing of elephant ivory [13]. The analysis of variations in stable isotope ratios (δ) at natural abundance levels offers potential for geo-locating the source of drugs that is production-process independent, if it can be established that ecological relationships exist between the isotopic composition of the compound(s) of interest and aspects of the environment. Such a possibility exists because of known isotopic fractionation events [14,15]. That is, the isotopic composition of plant material is fixed during biochemical synthesis and the observed isotopic variations reflect differences in metabolic processes or environmental conditions during growth. For instance, variations in carbon isotope ratios ($\delta^{13}C$) within a single plant taxa are associated with environmental growth conditions [16,17]. Plants grown under high humidity and/or high soil water availability may have $\delta^{13}C$ values up to 4–6‰ more negative than plants produced under low humidity or water-stressed conditions. Incorporating other stable isotopes into the analyses may allow further partitioning of geographic regions. For instance, hydrogen (δD) and oxygen ($\delta^{18}O$) isotope ratios of organic compounds record humidity–precipitation conditions during the growth period and plants may differ by more than 15‰ across different temperate regions [18,19]. Lastly, variations in plant nitrogen isotope ratios ($\delta^{15}N$) of 10‰ or more occur and record soil and microbial N_2 conditions [20].

Heroin is a semisynthetic product derived from morphine, which in turn is obtained from *Papaver somniferum*. *P. somniferum* is more commonly known as the opium poppy and is the source of the poppy seeds commonly used on bakery items and is the world's only source for medicinal morphine. All poppy species are C_3-photosynthesis plants and thus their $\delta^{13}C$ values are expected to be between −25‰ and −31‰, depending on environmental conditions. Illicit cultivation of the poppy occurs principally in Southwest Asia, Southeast Asia, Mexico, and South America [21]. Several recent studies have explored the possibility that carbon or nitrogen isotopic variation in morphine, and its derivative heroin, record specific geo-location information [22–26]. In each of these works the authors were optimistic about the potential use of isotope ratio analyses for the purposes of sample comparison and/or origin determination. However, each study was constrained by the difficulties associated with obtaining illicit samples across a wide geographical range, where the geographic origin was known with some certainty. Fortuitously we have access to a significant collection of illicit heroin and cocaine samples of reasonably certain geographic origin. This allows for the first time a broad assessment of variations in isotope ratio analyses at natural abundance levels for the purpose of origin determination of illicit cocaine and heroin samples.

2. Methods

A total of 76 heroin and 28 cocaine samples (see note later) were obtained from the U.S. Drug Enforcement Administration, Special Testing and Research Laboratory. No effort was made to purify an individual heroin sample before isotope ratio analyses.

Morphine samples were prepared by weighing an amount of heroin sample equivalent to 20 mg of morphine into a 14 ml conical test tube. To the tube was added 5 ml of 0.5 N NaOH and then the tube was maintained at 60°C for 30 minutes. After cooling, the solution was extracted with three 5 ml aliquots of chloroform. The chloroform was discarded and the aqueous solution was adjusted to pH 8 with 3 N HCl. Then the solution was exhaustively extracted with 5 ml aliquots of 3:1 methyl chloride and isopropyl alcohol. The total organic phase was subsequently dried with anhydrous sodium sulfate and then reduced to dryness under a stream of dry nitrogen at 60°C. The extraction was judged complete when no morphine could be detected in an individual extract. Starting with the fourth extract a 50 μl portion was removed from each organic phase extract for analysis. The 50 μl samples were first dried by passing them through a microcolumn containing anhydrous sodium sulfate. The columns were prepared by weighing approximately 100 mg of anhydrous sodium sulfate into a glass wool plugged Pasteur pipette followed by a wash with 1 ml of dry extraction solvent. A 50 μl sample was then added to the column followed by an additional 1 ml of dry extraction solvent. The approximate 1 ml of dried sample solution was then taken to dryness at 60°C under a stream of dry nitrogen. The analyses were accomplished by adding 100 μl of a 50:50 mixture of methylene chloride and *N,N*-Bistrimethylsilyl-trifluoracetamide to the dried sample, and then heating the sample for 30 minutes at 60°C. A 3 μl injection (30:1 split) was made from the sample mixture into a 15 m×0.25 mm DB-1 0.25 μm film thickness fused silica capillary column (J&W Scientific) interfaced to a Finnigan MAT GCQ mass spectrometer which was operated in the EI mode. When the molecule ion of di(t-rimethylsilyl)-morphine (*m/z* 429) could no longer be detected, the extraction was judged to be complete.

Individual samples (0.75–2.60 mg) were flash combusted on an elemental analyzer (Carlo Erba, model 1108 EA, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT model 252, Bremen, Germany) that was operated in the continuous flow mode. Combustion was in an O_2 atmosphere in a quartz reactor packed with Cr_2O_3 on alumina and $(Co_3O_4)Ag$ to form CO_2, N_2, NO_x, and H_2O. The gases were then passed through a copper furnace (650°C) to reduce NO_x to N_2. Water was subsequently removed by a $MgClO_4$, and finally N_2 and CO_2 were then separated by a GC column (4 m, 1/4-inch Poroplot Q) with reference gases inserted as pulses of pure standard gases. The carbon and nitrogen isotope composition as well as carbon and nitrogen contents were measured on the same sample. The C/N ratio served as an independent measure of sample purity.

Isotope composition is expressed in delta notation as the carbon or nitrogen isotope ratio (δ, units of ‰) relative to known standards (Pee Dee Belemnite for carbon and air for nitrogen):

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

where R is the ratio of the heavy to light isotopes. Precision of individual isotope ratio measurements as measured by 'Utah cabbage,' a long-term laboratory organic standard, were ±0.12‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

3. Results and discussion

On average, heroin samples from each of the four growing regions differed by as much as 2.4‰ and 3.1‰ in their $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Fig. 1 left). Overall, the $\delta^{13}C$ values for heroin were very light (negative), which is consistent with either plants growing in high humidity environments (usually seen in whole leaf values) and/or compounds resulting from strong secondary isotope fractionation events following photosynthetic carbon fixation [16,17]. With respect to geo-location potential, each of the major growing regions were distinguishable using parametric statistics (Fig. 1 left). The error bars span the 99% confidence interval and indicate only limited overlap in the $\delta^{13}C$ and $\delta^{15}N$ values of heroin samples from Mexico and South America. Southwest Asia and Southeast Asia samples were statistically distinct from each of the other regions. Considered as single samples instead of as population means, there was limited overlap in the $\delta^{13}C$ versus $\delta^{15}N$ parameter space of individual samples. Two-dimensional $\delta^{13}C$ versus $\delta^{15}N$ realms could be constructed which enclosed the observed isotope ratio variation for heroin samples from a geographic region. Specifically for individual heroin samples, only 2 of the 24 South American samples had any overlap with another realm, only 2 of the 20 Southwest Asia samples overlapped with another realm, none of the 26 Southeast Asia samples overlapped with another realm, and none of the 6 Mexico samples overlapped with another realm.

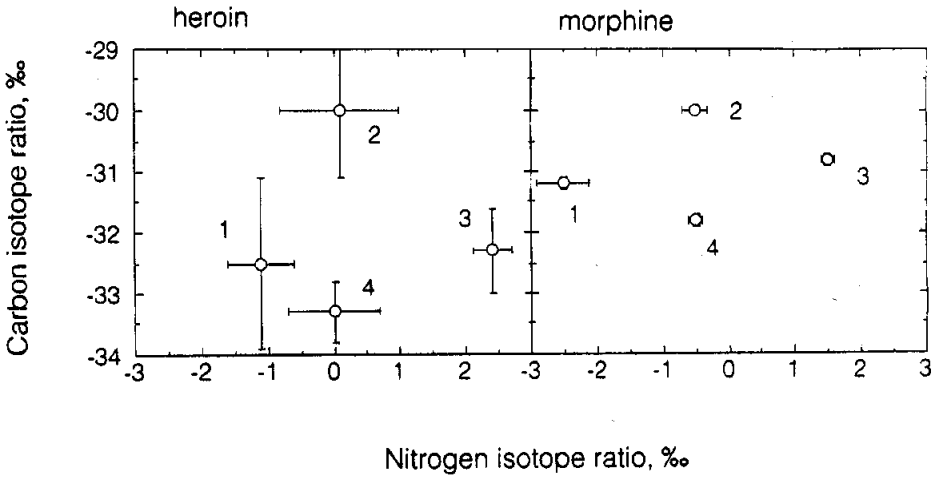

Fig. 1. Carbon and nitrogen isotope ratios of authenticated heroin (left plate) and associated morphine samples (right plate) originating from the major growing regions: Mexico (1), Southwest Asia (2), Southeast Asia (3), and South America (4). Error bars indicate 99% confidence interval.

Although the majority of the heroin samples selected for this work were not adulterated, that, of course, does not mean that these samples were pure heroin. Rather, all samples to some degree were contaminated with the usual assemblage of opium-related alkaloids with those originating from Southwest Asia most likely to contain appreciable quantities of the isoquinoline opium alkaloids. In Fig. 2, we plot the relationship between $\delta^{15}N$ values of the heroin and of the morphine derived from the heroin sample. The near 1:1 slope relationship indicates that most of the heroin samples had been nearly pure. For nearly all of these drug samples the C/N ratios of the heroin and extracted morphine were 19 ± 1 and 14.6 ± 1, respectively, which are the expected ratios of pure heroin and morphine.

Additionally, samples which originated from within Mexico are frequently cut heavily with sugars and other organic materials which are typically characterized as 'amorphous materials.' Although a large proportion of the samples had a heroin plus opium alkaloid content of at least 85% by weight, these contaminants could influence the isotopic composition of a bulk drug sample analysis. In Fig. 3, we have plotted the $\delta^{13}C$ values of morphine and of the associated heroin sample. The relationship between these two parameters was highly significant ($r = 0.667$, $P < 0.001$) for all heroin–morphine samples. Yet the slope of this relationship was far from 1:1. One likely possibility is that sugars from C_4-photosynthesis crops, such as corn sugar and cane sugar, had been added to a number of the heroin samples, making them heavier in ^{13}C than would have been expected. The $\delta^{13}C$ values of C_4-photosynthesis plants are typically -12 to 16 ‰, which is significantly heavier than that of C_3-photosynthesis plants, such as poppy [16,17].

Even if the samples were absolutely pure heroin, the $\delta^{13}C$ value may be expected to deviate from that of the plant. This is because heroin is derived by reacting morphine, the original plant product, with acetic anhydride, which then contributes 4 of the 21

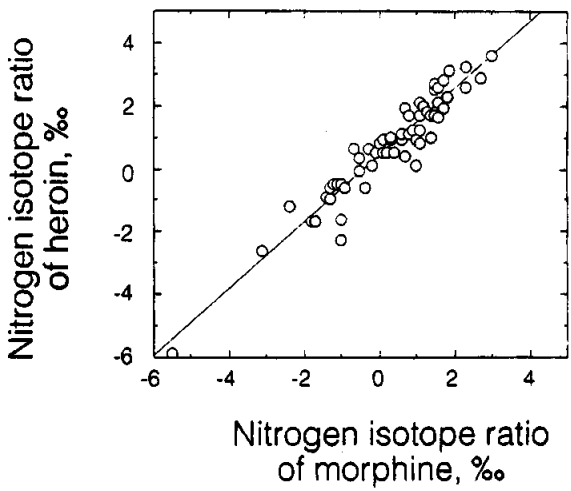

Fig. 2. The relationship between nitrogen isotope ratios of heroin and of the morphine derived from morphine samples. The slope of the relationship shown is $y = 1.05x + 0.40$ ($R^2 = 0.904$, $P < 0.001$).

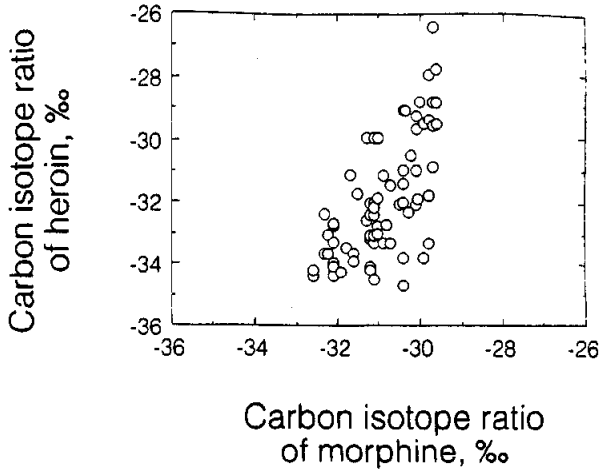

Fig. 3. The relationship between carbon isotope ratios of heroin and of the morphine derived from morphine samples. The correlation coefficient of the relationship shown is $r = 0.667$ ($P < 0.0001$).

carbon atoms in heroin. Others have shown quite conclusively that the acetyl moiety does alter the $\delta^{13}C$ value [26]. We measured the $\delta^{13}C$ values of acetic anhydride from two sources. The $\delta^{13}C$ values of acetic anhydride from Fisher Chemical (USA), Merck (Germany), and Merck-Schuchardt (Germany) were -20.23 ± 0.07‰, -31.12 ± 0.01‰, and -31.44 ± 0.01‰ (mean$\pm$1 SD), respectively. The extent to which the $\delta^{13}C$ values of acetic anhydride vary geographically or by manufacturer are unknown at present.

Each of the heroin samples was hydrolyzed to give morphine. The analyses of these morphine samples exhibited even clearer separations of their $\delta^{13}C$ and $\delta^{15}N$ values, differing by 1.4‰ and 3.5‰, respectively. Each of the four major growing regions was distinct isotopically (Fig. 1 right). The small error bars (99% confidence intervals) associated with each of the morphine data sets indicated that the major growing regions had significantly different and non-overlapping stable isotope ratio combinations. These isotope ratio data suggest heroin samples from each of the growing regions may be correctly geo-located possibly by analysis of the heroin molecule, but more completely through morphine analysis. Furthermore, differences in the $\delta^{13}C$ values of morphine and heroin may be useful in identifying the value of the acetic anhydride used in the morphine-to-heroin conversion.

Cocaine is derived from coca (*Erythroxylon coca*), a C_3-photosynthesis plant that is commonly grown in South American locations, such as Bolivia, Colombia, Ecuador, and Peru [27]. Cocaine samples obtained from each of the four growing regions differed by 0.6‰ and 7.1‰ in their $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Fig. 4). Although the number of cocaine samples were fewer than for heroin analyses, the four geographic regions were isotopically distinct, indicating that geo-location for cocaine may also be possible using stable isotope ratio analyses.

The natural range in both $\delta^{13}C$ and $\delta^{15}N$ values of morphine and cocaine reflect variations in some aspect(s) of the environmental growth conditions. Ecological features

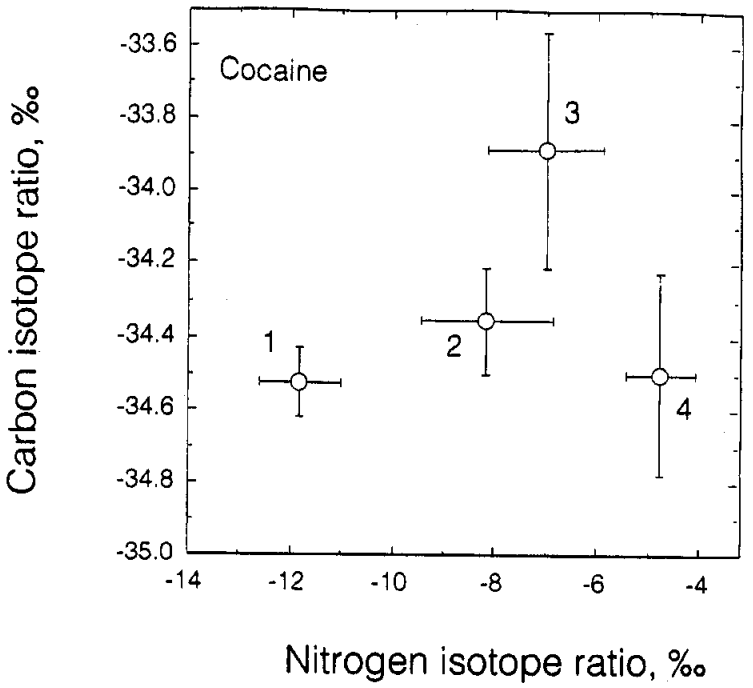

Fig. 4. Carbon and nitrogen isotope ratios of authenticated cocaine samples originating from the major growing regions in South America: Bolivia (1), Peru (2), Ecuador (3), and Colombia (4). Error bars indicate ±1 standard error.

that have been associated with variations in $\delta^{15}N$ values of plants include precipitation, land-use history, and fertilization [18,20]. Too little is known of the specific growth conditions from which our samples were derived to be able to quantify environmental relationships further. However, it is likely that whole-plant studies from known field sites will clarify these patterns. From the broad regional-climate data available, we know that the variations in $\delta^{13}C$ values of morphine are consistent with known differences in humidity conditions among the four geographic regions.

It is interesting to note that the isotope ratios of both heroin and cocaine differ significantly from that of bulk plant tissues [18,20]. Specifically, the $\delta^{13}C$ and $\delta^{15}N$ values are each at least 3–8‰ more negative than typically observed for whole tissue materials. Whether or not all alkaloids within plant tissues differ to the same extent is largely unknown, but it is known that natural and synthetic alkaloids differ in detectable ways [28,29].

The analysis of multiple isotope ratio combinations holds potential as a useful approach in geo-locating the origins of specific biological molecules, such as heroin, cocaine, and other materials of economic and forensic interest. We have shown for the first time that the current major geographic origins for both heroin and cocaine can be identified on the basis of natural variations in isotope ratios, which are related to variations in habitat environmental parameters. The δD and $\delta^{18}O$ values of cocaine and

morphine remain unexplored and may allow further geographic partitioning, because such variations are related to precipitation–humidity conditions [17–19].

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