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Isotopic Analysis of ^{13}C as a Tool for Comparison and Origin Assignment of Seized Heroin Samples*

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ABSTRACT: The applicability of isotopic analysis of ^{13}C for forensic purposes has been discussed in the case of heroin. The obtained results showed first that morphine acetylation induces an isotopic fractionation, so that the ^{13}C enrichment of diacetylmorphine depends on both geographical origin of the sample and source of acetic anhydride used by the drug trafficker. That measurement can then be of great interest in the determination of common-batch samples (tactical intelligence purpose). Second, diacetylmorphine deacetylation has also been studied and it appeared that this deacetylation allows to relieve the enrichment from the acetylation-induced part. Therefore, measuring morphine ^{13}C enrichment, from deacetylated heroin samples, can be useful for determining the geographical origin of the samples (strategic intelligence purpose). Moreover, measuring both diacetylmorphine and morphine ^{13}C enrichments may help identify the source of acetic anhydride used by the drug trafficker, so that the fraudulent laboratory where the morphine samples have been acetylated.

KEYWORDS: forensic science, drugs of abuse, heroin, isotopic analysis, origin assignment

Drug abuse and particularly heroin addiction has escalated dramatically in recent years. In response to this global threat, the United Nations has proclaimed the period between 1991 and 2000, the United Nations "Decade for Eradicating Drug Abuse," as a time for intensifying and sustaining international and national efforts in the fight against Drug Abuse. As a key part of drug enforcement, identification of sources of supply and distribution patterns of illicit drugs is of major interest.

In that aim, the basis of comparative analysis for strategic and tactical intelligence purposes is the concept of "drug signature" (1) which can be divided into two approaches.

The first and former one is the "impurities profile" technique. It deals with the characterization of manufacturing impurities and by-products in illicit drugs so as to determine the geographical origin of the drug, when applicable, and to compare different drug seizures for common source determination. Some studies about these trace level chemical analyses have already been reported on cocaine (2–5), heroin (6–10), amphetamine-type compounds (11–18) and phencyclidine (19).

Nevertheless, because impurities are parameters extrinsic to the drug of concern, they can be intentionally or unintentionally altered in the storage and distribution processes. An alternative approach is then the "stable isotope ratio" technique (20). It deals with the determination and comparison of naturally abundant stable isotope ratios of the drug sample or the drug molecule itself. The isotope content of a plant, and of its constituents, is characterized by its photosynthetic cycle and the metabolic pathways of the fixation of carbon, nitrogen, oxygen, hydrogen. Moreover, environmental conditions such as humidity, temperature, photoperiod, isotope composition of ambient carbon dioxide in which the plant has grown, can also modify this isotope content. These variations can be estimated by the measurement of isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$). Because isotope ratio is an intrinsic parameter of the drug itself, partial degradation or addition of foreign materials during the storage and distribution processes would not alter the analytical result. For instance, in 1979, Liu et al. tried to obtain isotopic information on the geographical origin of Cannabis by measurements of carbon isotope ratios from both leaves and flowers of the plant (21). Moreover, the isotopic analysis of synthetic drugs such as methylamphetamines (22) allowed the comparison and discrimination between the origin of various samples.

For heroin, a preliminary study showed significant differences between samples from disparate geographical origins (Turkey, Thailand, India, and Pakistan) via isotopic analysis of ^{13}C (23). Nevertheless, on that point, it is necessary to remember that diacetylmorphine is made of one alkaloidic part (morphine) and two acetyl substituents (afforded by the synthesis). Some differences in ^{13}C enrichment are therefore due to the alkaloidic part by the geographical origin, whereas the remainder is due to the acetyl part according to the source of the acetylating agent used. In this article, the applicability of the isotopic analysis of diacetylmorphine for forensic purposes is discussed, by determining the isotopic fractionation due to morphine acetylation.

Materials and Methods

Chemical Reagents

- Diacetylmorphine base (DAM_b), diacetylmorphine hydrochloride (DAM_{HCl}), morphine base (M_b) (Sanofi-Francopia, Gentilly, France).
- Acetic anhydride extra pure (Merck, Darmstadt, Germany), pyridine (Interchim, Montluçon, France).

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• Anhydrous sodium carbonate (Rhône-Poulenc, France), anhydrous sodium sulfate and potassium dihydrogenophosphate (Merck, Darmstadt, Germany).

• Chloroform, ethyl alcohol absolute, acetonitrile, acetone, methyl alcohol, hydrochloric acid 37% (Carlo Erba, Milan, Italy), 2-propanol (Merck, Darmstadt, Germany).

High Performance Liquid Chromatography (HPLC)

In order to determine the synthesis yields, HPLC was used. HPLC analysis was carried out using a HP 1090 liquid chromatograph coupled with a HP 1040A photodiode array. The chromatographic column was a HP ASAHIPAK ODP-50: L = 125 mm, ID = 4 mm, granulometry = 5 μm.

The working conditions were: Isocratic mode, flow rate: 0.8 mL/min, injection volume: 5 μL, detection: 230 nm, mobile phase: Acetonitrile-K_2HPO_4 0.015 mol/L (25:75, v/v), and temperature: 30°C.

Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS)

The chromatographic separation was achieved on a HP 5890 series II gas chromatograph and isotopic measurements on a ISOCHROM OPTIMA VG ISOTECH. A capillary column was used (DB-1, L = 30 m, ID = 0.25 mm, film thickness = 0.25 μm). Helium was the carrier gas and the pressure at the head of the column was set at 110 kPa. Samples (1 μL) were injected according to the splitless mode (valve time: 0.8 min). The injection port temperature was set at 285°C and the oven temperature program was as follows:

for morphine samples: The oven temperature was raised from 180°C (1 min) to 250°C (13 min) at 40°C/min, then to 300°C (9 min) at 40°C/min, for diacetylmorphine samples: The oven temperature was raised from 160°C (1 min) to 260°C (20 min) at 40°C/min.

After chromatography, the GC effluent was sent either to the flame ionization detector (FID) or to the isotope ratio mass spectrometer (IRMS) via the combustion interface. That interface consisted of a quartz capillary furnace packed with copper oxide granules, set at 840°C. Organic compounds were combusted to carbon dioxide and water. Water was condensed using a cryogenic trap held at −100°C ± 1°C, after which carbon dioxide was introduced into the mass spectrometer for $^{13}C/^{12}C$ analysis at m/z 44, 45, and 46. Because the natural variations of carbon isotope ratios are generally small, the results are conventionally expressed in terms of relative difference with respect to a reference CO_2 derived from PDB (calcium carbonate—a fossil of *Belemnitella Americana* from the Peedee formation in South Carolina—$^{13}C/^{12}C$ ratio = 0.011,1237), using the delta notation (24):

$$\delta^{13}C‰ = 1000 \times [(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{reference}]/(^{13}C/^{12}C)_{reference}$$

Acetylation Procedure

1.6 mg of morphine base is dissolved in 300 μL of an acetic anhydride-pyridine mixture (1:1, v/v) and slightly stirred in a 2 mL vial for 1 min. This solution is then heated for 30 min at 50°C, and stirred two times for 1 min during heating. After heating, the solution is stirred one more time for 1 min and left 24 h at room temperature, protected from light. The solution is then evaporated to dryness at 40°C, under a nitrogen stream, and the residue frozen. It is dissolved into 500 μL of ethyl alcohol before analysis. The acetylation yield is 95%, as determined by HPLC.

Deacetylation Procedure

Diacetylmorphine Hydrochloride—A mass of sample containing 1.5 mg of diacetylmorphine hydrochloride is dissolved in 7.5 mL of water and 7.5 mL of 1 mol/L sodium carbonate solution in a 45-mL flask. After homogenization, the light protected flask is placed in a water-bath at 37°C during 5.5 h. The solution is then cooled before pH adjustment to 8.5 by addition of a hydrochloric acid solution (18%). Morphine is extracted with 200 mL of a chloroform-isopropanol mixture (8:2, v/v). The organic layer is dried over Na_2SO_4, and left 24 h in the refrigerator (5°C). Thereafter, the solution is filtered through a Whatman phase separator, and concentrated to 2 mL at 45°C under a nitrogen stream. It is then transferred to a 10-mL vial. The 45-mL flask is washed with chloroform (2 by 2 mL) and the combined organic layers added in the vial. The corresponding mixture is evaporated to dryness, at 45°C under a nitrogen stream, and frozen. The residue is dissolved in 500 μL of ethyl alcohol before analysis.

Diacetylmorphine Base—A mass of sample containing 1.3 mg of diacetylmorphine base is dissolved in 500 μL of acetone. After stirring for 1 min, 300 μL of hydrochloric acid (0.1 mol/L) are added. After stirring one more time for 1 min, the mixture is evaporated to dryness, at 45°C under a nitrogen stream. The obtained residue is dissolved into 7.5 mL of water and 7.5 mL of a sodium carbonate solution (1 mol/L) in a 45-mL flask. Then, the procedure is the same as for a diacetylmorphine hydrochloride sample. For both procedures, the deacetylation yield is 97%, as determined by HPLC.

"Pirouette" Procedure

"Pirouette" is herein defined as the acetylation of morphine into diacetylmorphine followed by the total deacetylation of the obtained diacetylmorphine. The aim of that procedure is to compare the ^{13}C enrichments of initial and final morphine molecules. The procedure is then divided into two consecutive manipulations:

Morphine acetylation, same procedure as the one previously described above; Diacetylmorphine deacetylation, same procedure as the one previously described for diacetylmorphine base above. The "pirouette" yield, determined from the quantitative ratio (final morphine/initial morphine), is 95%, as determined by HPLC.

Results and Discussion

Acetylation of Morphine

The complete acetylation of morphine into diacetylmorphine has been performed in order to measure the potential corresponding isotopic fractionation. The results are gathered in Table 1. To determine the enrichment of diacetylmorphine from the enrichment of morphine, the following equation is applied, if no isotopic fractionation occurs during acetylation:

$$A + B \rightarrow C$$

$$n_C \times \delta_C = n_A \times \delta_A + n_B \times \delta_B$$

n_i = number of carbon atoms of i molecule

$\delta_i = \delta^{13}C‰$ of i molecule

TABLE 1—*^{13}C enrichments of morphine (M_b) and synthesized diacetylmorphines (DAM).*

Compound	M_b	DAM Acetylation 1	DAM Acetylation 2	DAM Acetylation 3	DAM Acetylation 4	DAM Acetylation 5
$\delta^{13}C‰$	−30.54	−33.60	−33.63	−33.61	−33.70	−33.66
$\delta^{13}C‰$ (DAM)		−3.29	−3.32	−3.30	−3.39	−3.35
$\delta^{13}C‰$ (M_b)						

Therefore, considering morphine acetylation (Fig. 1):

$$21 \times \delta_{\text{diacetylmorphine}} = 17 \times \delta_{\text{morphine}} + 4 \times \delta_{\text{anhydride}} (\delta_{\text{anhydride}} = -29.33\ \delta‰)$$

$$\text{so } \delta_{\text{diacetylmorphine}} = \delta_{\text{theoretical}} = -30.31\ \delta‰$$

There is a significant difference (Student test, $p < 0.025$) between the theoretical and experimental values. Furthermore, this difference is constant and reproducible, thus it is possible to conclude that the isotopic fractionation due to the acetylation is reproducible and, in our case, its value is: −3.33 δ‰.

Isotopic Analysis of Seized Heroin Samples

Thirty-one seized heroin samples have been analyzed. Their geographical origin was given by the customs or police department which seized them. It is probable that many origins are doubtful (especially African ones) and it is of major importance to keep in mind that the authentics used herein are not certain.

The mean ^{13}C enrichment of all samples are reported in Table 2 and shown in Fig. 2. The overall intrinsic reproducibility of GC-IRMS, taking into account all variations that can occur during isotopic measurement, is given as 0.3 δ‰ by the instrument manufacturer:

First, it is interesting to focus on common-batch samples (encircled points in Fig. 2, origins marked with the (*) symbol in Table 2). With a high probability, these samples have the same "story," i.e., same geographical origins and same synthesis routes. Their enrichments are compared with each other by using a two-sample *t*-test (Student test, $p < 0.025$) and the conclusion is that there is no significant difference between them. Second, looking at the whole results, it is noticeable that even within a common origin category, the measured values of isotopic enrichments are scattered. These results suggest that the utilization of the ^{13}C analysis of diacetylmorphine may be unsuitable for the origin assignment of heroin samples. As seen before, the isotopic fractionation (around −3 δ‰), induced by the acetylation of morphine, represents a consistent part of the differences of ^{13}C enrichments measured between heroin samples (Fig. 2). For instance, a same morphine sample acetylated with two different acetic anhydrides, i.e., from

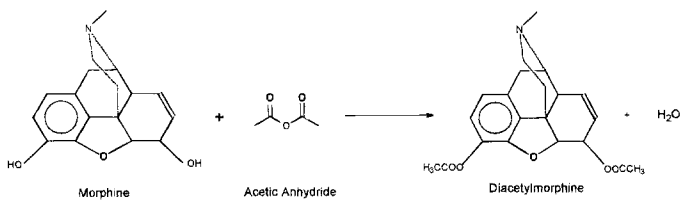

FIG. 1—*Acetylation of morphine with acetic anhydride and pyridine.*

TABLE 2—*^{13}C enrichments measured from heroin samples (DAM) and deacetylated heroin samples (M).*

Origin	$\delta^{13}C‰$ (DAM)	$\delta^{13}C‰$ (M)	$\delta^{13}C‰$ (DAM)—$\delta^{13}C‰$ (M)
India	−29.41	−29.58	0.17
India	−33.09	−29.83	−3.26
India	−28.06		
India	−31.31	−29.31	−2
India	−30.26	−29.57	−0.69
Africa	−29.41	−29.45	0.04
Africa	−34.97	−29.98	−4.99
Lebanon	−32.12	−28.66	−3.46
Lebanon	−30.92	−28.75	−2.17
Lebanon*	−31.42	−28.94	−2.48
Lebanon*	−31.31		
Lebanon*	−31.29		
Lebanon*	−31.33	−28.93	−2.40
Lebanon*	−31.34		
Turkey	−32.05	−30.09	−1.96
Turkey	−31.45	−29.72	−1.73
Turkey*	−33.38		
Turkey*	−33.16		
Turkey*	−33.29		
Turkey	−32.73	−29.64	−3.09
Thailand	−32.25	−30.45	−1.80
Thailand	−31.97	−30.21	−1.76
Thailand	−33.17		
Thailand	−32.72	−30.18	−2.54
Thailand	−34.50		
Thailand	−35.38	−30.54	−4.84
Pakistan	−28.78		
Pakistan	−32.79		
Pakistan	−29.45		
Syria	−32.94		
Syria	−31.78		

*Common-batch samples.

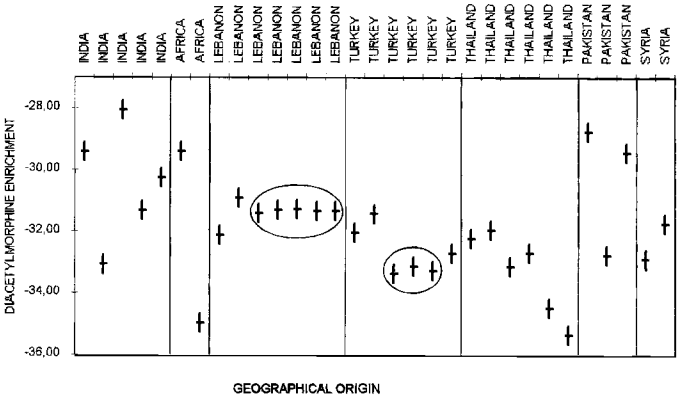

FIG. 2—*^{13}C enrichments of diacetylmorphines measured from heroin samples.*

different sources, will probably give two diacetylmorphine samples with different ^{13}C enrichments. This difference could be assigned, by mistake, to the samples' geographical origin, whereas it is actually only due to the synthesis.

Nevertheless, there is an interesting application of the ^{13}C analysis of diacetylmorphine: Using it for tactical intelligence purpose. As different geographical origins or different acetylating agents induce different ^{13}C enrichments, the isotopic analysis of diacetylmorphine could be a complementary technique to the impurities profile technique so as to compare heroin samples for court evidence and determine which ones are common batch samples.

"Pirouette"

The complete acetylation of morphine has been performed, and the obtained diacetylmorphine in turn deacetylated back to morphine. The results are gathered in Table 3.

There is no significant difference (Student test, $p < 0.025$) between standard and "pirouette" made morphine values. Therefore, the isotopic fractionation due to diacetylmorphine deacetylation is reproducible and its value is the same as the one corresponding to morphine acetylation, that is to say, in our case: -3.33 δ‰. That means that diacetylmorphine deacetylation relieves the isotopic enrichment from the acetylation. Consequently, diacetylmorphine deacetylation could be used to enhance strategic intelligence purpose, as it gives access to the precursor ^{13}C enrichment (morphine) which only reflects the environmental conditions in which the opium poppy has grown, i.e., the geographical origin of the sample.

Isotopic Analysis of Deacetylated Seized Heroin Samples

Seventeen seized heroin samples have been deacetylated, and the so-obtained morphine samples analyzed. Their mean ^{13}C enrichments are reported in Table 2 and shown in Fig. 3. First,

TABLE 3—*^{13}C enrichments of morphines obtained with the "pirouette" procedure.*

Compound	M_b	M "pirouette" 1	M "pirouette" 2	M "pirouette" 3
$\delta^{13}C$‰	−30.54	−30.37	−30.42	−30.28
$\delta^{13}C$‰ (M) $\delta^{13}C$‰ (M_b)		0.17	0.12	0.26

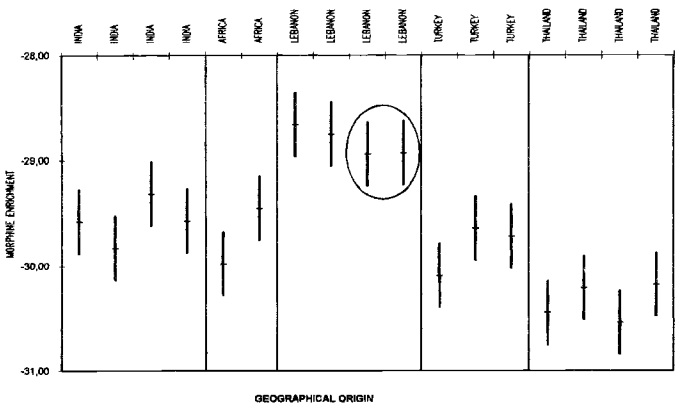

FIG. 3—*^{13}C enrichments of morphines measured from deacetylated heroin samples.*

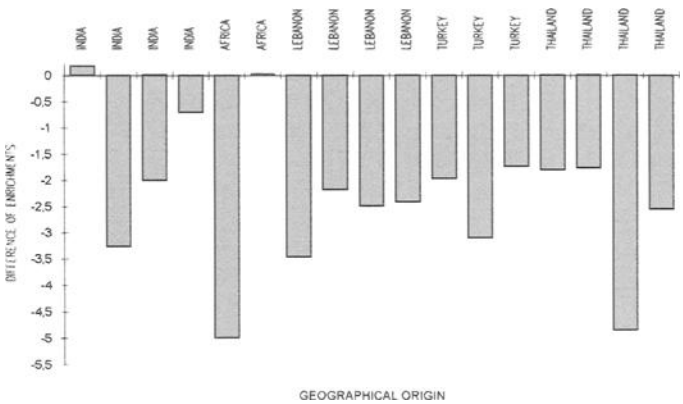

FIG. 4—*Difference of ^{13}C enrichments between diacetylmorphine and morphine.*

for common-batch samples (encircled points in Fig. 3, origins marked the (*) symbol in Table 2), and more generally for common origin samples, there is no significant difference between the ^{13}C enrichments (Student test, $p < 0.025$). It confirms the usefulness of deacetylating diacetylmorphine, i.e., recovering the ^{13}C enrichment of the natural precursor which is only linked to the geographical origin of the sample. However, it is difficult to differentiate some samples from various origins because their enrichment values are too close. This may be due to the fact that the origins are inaccurate, or, perhaps, indicates that the ^{13}C enrichment of the molecule is not informative enough to discriminate samples from different geographical origins. For morphine, the enrichments range is only 3 δ‰, and 0.6 δ‰ is necessary to differentiate two values; therefore, principal components analysis (PCA) could be the proper means to improve the discrimination on the basis of various criteria, not only ^{13}C enrichment of morphine, but also quantitative ratio acetylcodeine/(monoacetylmorphine + diacetylmorphine). However, to be conclusive, that multivariate approach would require undoubted heroin authentics.

Difference of Isotopic Enrichments Between Diacetylmorphine and Morphine

For seventeen seized heroin samples, both diacetylmorphine and morphine enrichments have been determined, the latter one being measured from the deacetylated samples. The so-obtained differences are reported in Table 2 and shown in Fig. 4. These differences are linked to the isotopic enrichment of the acetylating agent used by the drug trafficker. Therefore, it is necessary to carry out numerous acetylations with different acetylating agents, and to input the obtained isotopic differences (morphine-diacetylmorphine) in a database. In the near future, that database will may be a good means to type the fraudulent laboratory in which the morphine samples were acetylated.

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