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Geographic Origin Determination of Heroin and Cocaine Using Site-Specific Isotopic Ratio Deuterium NMR

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ABSTRACT: SNIF-NMR® (Site-specific natural isotopic fractionation measured by deuterium NMR) was employed on 36 heroin samples from seven different known origins, and two cocaine samples from two different known origins. Heroin has two "synthetic" deuterium labeled sites (the two acetyls from acetic anhydride, each representing three equivalent nuclei) and 15 "natural" deuterium labeled sites (originating from the morphine produced in the opium plant). The "natural" sites have the potential for determining geographic location of the original opium plant, while the "synthetic" sites could assist in giving information about the commercial source of acetic anhydride used to convert morphine to heroin. Cocaine has 15 "natural" deuterium labeled sites. This study shows that SNIF-NMR® has some use in determining the geographic origin of heroin and also has good potential for determining the geographical origin of cocaine.

KEYWORDS: forensic science, deuterium nuclear magnetic resonance, SNIF-NMR, stable isotope analysis, heroin, cocaine, geographical origin, substance abuse, chemistry

Heroin and cocaine are illicitly produced drugs (see Fig. 1 for molecular structures) that are abused throughout the world. Heroin originates from the acetylation of morphine isolated from latex collected from the opium poppy. Poppy cultivation and heroin production occur in four major regions: South America, Southeast Asia, Southwest Asia, and Mexico (Fig. 2*a*) (1). Cocaine is a naturally occurring alkaloid which is extracted from coca leaf. Coca cultivation primarily occurs in Peru, Bolivia, Colombia, and Ecuador (Fig. 2*b*) (2). Determining the geographical origin of these two drugs is of major intelligence importance (3).

Currently, high-resolution chromatographic methods are utilized to examine impurity profiles and thereby determine processing origin (which is closely associated with specific geographical regions). Existing methodologies rely on identifying specific processing impurities and minor alkaloids present in illicitly produced heroin (1,4–6) or cocaine (2,7–10). This has worked very well in most cases; however, for "highly refined" samples, where the impurities are either absent or present in only very low amounts, this method is often inconclusive.

Stable isotopic ratio studies have been performed on many natural products to determine origin, adulteration, or authenticity. The two most common isotopic ratio methods are isotopic ratio mass spectrometry (IRMS) (11–13,18,19,22–33,38,40) and site-specific isotopic ratio deuterium nuclear magnetic resonance spectrometry (DNMR) (DNMR:11–23,39; ^{13}CNMR:15,17). IRMS looks at the molecule's global isotope ratio of the various isotopes of the element under investigation (carbon 13 vs. 12, nitrogen 15 vs. 14, oxygen 18 vs. 16, deuterium vs. hydrogen, etc.). Preliminary studies using this method have already been performed to determine its feasibility to determine the geographical origin of heroin and cocaine (35,37). The advantages of IRMS are its short experimental time frame and high sensitivity, requiring only a few milligrams of purified sample. The disadvantages are that only one data point is obtained per element (that being the relative enrichment of a specific isotope versus a standard), and separate analyses are required for each element.

SNIF-NMR® has also been used for many years for determining geographic origin, adulteration and authenticity of many natural products (wine, tobacco, fruit juices, etc.). This patented method obtains the deuterium NMR spectrum of a purified sample (95 to 100% pure) of the compound under investigation and determines the quantitative value of the deuterium enrichment versus an internal standard. Unlike IRMS, however, DNMR has the potential of obtaining quantitative values for every deuterium labeled site (in the case of heroin (Fig. 1*a*) the NCH_3 and 14 numbered sites); therefore, many more data points are available to help determine the geographic origin of the sample. In addition, the "synthetic" deuterium sites (i.e., the two acetyl groups in heroin) can be studied independently (whereas in IRMS, they would have to be hydrolyzed off before analysis, lest they introduce an artificial enrichment of the deuterium, carbon-13 and oxygen-18 levels). The disadvantages of this method arise from the fact that deuterium is a relatively insensitive nuclei and is present in only low relative abundance; therefore, large sample sizes and long experiment times are required, especially for large molecules like heroin. In addition, deuterium-NMR spectra are not well resolved, and quantitation of overlapping peaks can be problematic.

This study examined 36 heroin samples from seven known geographic origins, and two cocaine base samples of known origin. The results suggest that this method can be successfully used to assist in the determination of the origin of heroin; however, it appears at this time that it cannot determine origin on its own. In contrast,

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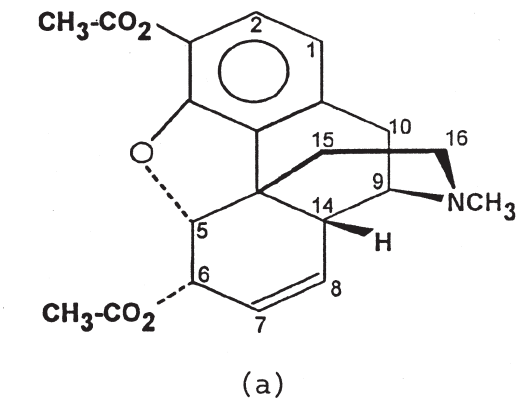

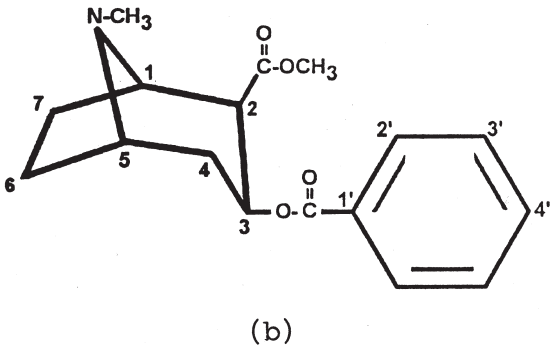

FIG. 1—*Molecular structures:* (a) *heroin,* (b) *cocaine.*

the results from the two cocaine base samples are very promising and warrant further investigation.

Equipmental Procedures

Instrumentation

A Bruker AM-500 NMR, equipped with a 10 mm broadband probe, operating at 76.77 MHz for deuterium, with proton decoupling, and a fluorine-19 lock (Bruker NMR Instruments, Germany), was used for all samples. Instrument set up and analysis of resulting spectra was done using SNIF-NMR® techniques (11–14,16,18,20–22,31,39). Sample preparation was accomplished using a Waters Delta Prep 4000 Preparative HPLC equipped with a Waters 991 Photodiode Array Detector (Waters Corp., Milford, MA), using either a Zorbax SIL 21.2 mm inside diameter (ID) ×

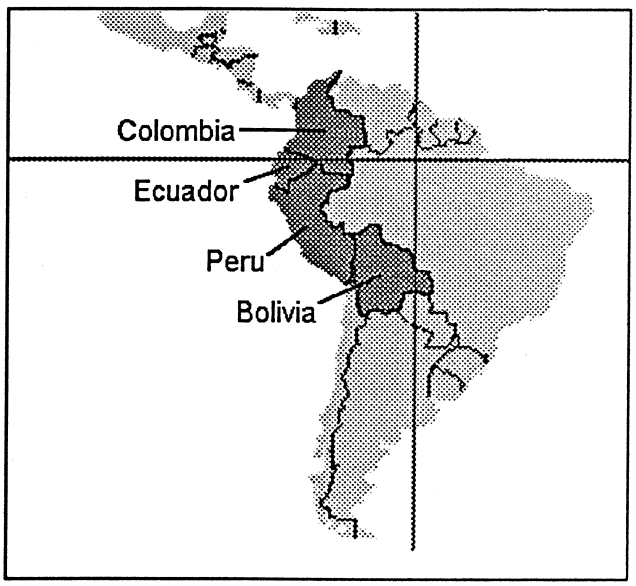

FIG. 2*b*—*Major cocaine production regions of the world.*

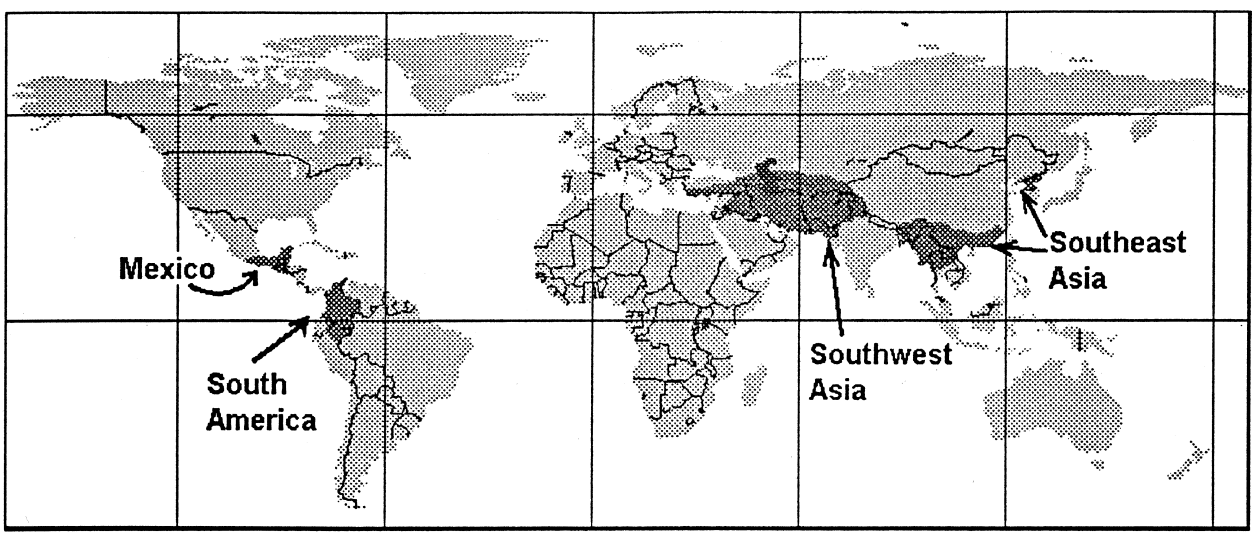

FIG. 2*a*—*Major heroin production regions of the world; South America, Mexico, Southwest Asia, and Southeast Asia.*

25 cm Preparative HPLC column (MAC-MOD Analytical, Chadds Ford, PA) or a Waters Prep Pak Cartridge, Bondapak C18, 47 mm ID × 300 mm, 15 to 20 μm, 125 Å Preparative HPLC column (Waters Corp., Milford, MA). Fractions were collected using a Foxy 200 X-Y Sample Collector (Isoco, Lincoln, NE). Evaporation of organic solvents employed a Büchi RE 111 and RE 114 Rotavapors, with Büchi B-461 and B-480 Water Baths (Büchi, Switzerland), respectively. Freeze drying was accomplished using a homemade freeze dryer with an Alcatel model #2033 high vacuum pump (Alcatel, France) and a freeze dryer manifold/solvent collector by Labconco (Kansas City, MO). Detection of morphine, O3- or O6-monoacetylmorphine, heroin, and related opium alkaloids in the HPLC fractions was accomplished using a Hewlett Packard HP Series II Plus 5890 Gas Chromatograph equipped with either a flame ionization detector or a Hewlett Packard HP 5971 Series Mass Selective Detector (MSD) (Hewlett-Packard, Palo Alto, CA).

Chemicals and Samples

The following HPLC grade solvents (Burdick and Jackson, Muskegon, MI) were used for sample purification: chloroform, methanol, methylene chloride, and hexane. HPLC grade water was obtained using the Milli-Q Water System (Millipore, Milford, MA). The processing of heroin and opium used analytical grade ammonium hydroxide (Mallinckrodt, St. Louis, MO), anhydrous granular sodium sulfate (Baker Chemical, Phillipsburg, NJ), sodium acetate (Aldrich Chemical, Milwaukee, WI), and acetic anhydride (source unknown). Hexafluorobenzene (SDS, Pépin, France) was used for NMR lock signal and chloroform (SDS, Pépin, France) as the solvent. The preliminary heroin study used nitromethane (SDS, Pépin, France) as the internal standard. Methenamine (SIGMA, St. Quention Fallavier, France) was used as the internal standard for the final heroin study and the preliminary study of cocaine.

Heroin (base and hydrochloride) and cocaine base came from seizures made either at or close to the actual source of production. Opium samples came from the research collection at the DEA Special Testing and Research Laboratory, and were obtained during a previously completed worldwide opium study where opium latex samples were obtained at the actual sites of poppy cultivation.

Purification of 19 Heroin Samples of Known Origin

All samples were purified to 95% or better (based on GC-FID) using a modified normal phase HPLC procedure by Wittwer (42). The heroin sample (heroin base equivalent weight of 135 milligrams[3]) was dissolved in chloroform (15 mL) with sonication, then filtered. A saturated aqueous solution of sodium bicarbonate (15 mL) was combined with the resulting solution in a separatory funnel, shaken, and the chloroform isolated and passed through a column of dry sodium sulfate and collected. Two new 15 mL volumes of chloroform were added to the remaining aqueous solution, shaken, isolated, dried with sodium sulfate, and combined with the first fraction. The solution of combined chloroform extracts (containing the heroin base and other impurities) was rotevapped to dryness, dissolved in the mobile phase, filtered and injected on the prep HPLC.[4] The compound that caused the greatest problems with respect to isolating pure heroin was acetylcodeine. A Zorbax SIL column (1 in. [2.54 cm] ID prep silica) was employed, using an isocratic mobile phase of 75% hexane and 25% mixture (dry chloroform-methanol-water-conc. ammonia [800 + 200 + 4 + 1]).[5] The flow rate was 40 mL/minute, with fraction collection every 30 s. Acetylcodeine eluted at about 10 min with some tailing into the heroin peak (which began at 12 min and ended about 2 min later). At 20 min after injection, a step gradient to 100% methylene chloride was employed to clean the column of remaining compounds. A 15 min equilibrium was performed prior to the next injection. Fractions were tested for heroin using GC-FID (DB-1, 30 m column, 0.25 mm ID, 0.25 μm film, 200°C (1 min hold), 12°C/min, 280°C (7 min hold)). The entire heroin peak, regardless of purity, was collected to prevent isotopic fractionation (40). Each heroin fraction was rotevapped to dryness, taken up in methylene chloride, dried with sodium sulfate to remove residual water and rotevapped to dryness again. The color of the resulting crystals varied from white to yellow.

Processing and Purification of 17 Opium Samples to Heroin Base

A 30 g sample of dried opium was powdered and added to an Erlenmeyer flask containing 150 mL methanol. The methanol was brought to near boiling and kept at that temperature for 30 min. The resulting solution was filtered, and the filtrate rotevapped (50°C water bath) to dryness. The filtered opium residue was again added to another volume of fresh methanol and the procedure repeated for a total of five times. The combined brown, gummy, methanol solubles (which included morphine) were dried further using a high vacuum pump. If not subjected to prep HPLC immediately, the sample was placed in a refrigerator to prevent degradation.

The HPLC mobile phase consisted of one bottle of a mixture of water-glacial acetic acid (5910 + 90)[6] and another bottle of methanol. The sample was added to 300 mL water-glacial acetic acid and stirred for 30 min. The resulting solution was filtered and suction injected onto a Waters Prep Pak C18 Bondapac reverse phase column with UV detection after the column to monitor compound elution. The pump operated at 90 mL/min with the following program: 10 min isocratic at 2% methanol/98% aqueous acetic acid followed by a 10 min linear gradient to 10% methanol/90% aqueous acetic acid, then a 5 min linear gradient to 100% methanol with 25 min hold. Fractions were collected every 15 s. Equilibration was established using a 5 min linear gradient returning to 2% methanol/98% aqueous acetic acid followed by a 15 min hold at these conditions.

Analysis of each fraction was accomplished by taking 20 drops of the solution and adding it to a vortex test tube containing 1 mL chloroform and 1 mL saturated aqueous sodium bicarbonate solution; this was vortexed for 30 s and the chloroform layer removed, passed through dry sodium sulfate to remove moisture, and an aliquot injected on a GC-FID or GC-MSD (using single ion monitoring, SIM, at the base peak mass for morphine; $m/z = 285$). All fractions containing morphine were collected,[7] combined, mixed, and freeze dried. The resulting morphine acetate ranged in weight from 2 to 5 g with a range of colors and textures (brilliant yellow crystals to yellowish-brown or green gum).

[3] This method gave a 75% recovery of the heroin, yielding 100 mg.

[4] Two high purity Southeast Asian hydrochloride samples were not subjected to this aqueous bicarbonate extraction method. Instead, the mobile phase (10 mL) containing 20 drops diethylamine was added to each sample and sonicated. This solution was clear, and was therefore directly injected onto the HPLC.

[5] The initial ammonia concentration in the mobile phase was 1 mL. This was eventually increased to 2.5 mL to improve peak shape.

[6] Ammonia was added to some runs as an amine modifier to improve peak shape and resolution.

[7] Morphine retention time from 9 to 14 min, codeine from 15 to 20 min.

Sodium acetate (4 g, dried) was added to a condenser flask containing the morphine acetate (2 to 5 g). Acetic anhydride (25 mL) was added, and the flask was stirred and heated to near boiling over 20 min. After 20 min, the solution turned from dark brown to a deep purple color, and the flask was removed and cooled. The solution was tested to see if the morphine was completely converted to heroin as follows: one drop of the solution was added to 1 mL saturated aqueous sodium bicarbonate solution and 1 mL chloroform in a vortex test tube and vortexed for 30 s. The chloroform layer was isolated and dried with sodium sulfate, and an aliquot injected on a GC-FID. If no or only minimal morphine or monoacetylmorphine was detected, 50 mL water was added to the reaction mixture to quench the residual acetic anhydride. The resulting solution was stirred for 5 min or more, then 75 mL mobile phase (5% methanol/95% aqueous acetic acid (5910 water + 90 glacial acetic acid)) was added with stirring. When the solution had cooled to room temperature, it was filtered and injected on the prep HPLC, using the same column and solutions as above, with a linear gradient of 5 to 100% methanol over 40 min; fractions were collected every 15 s. Heroin came off beginning at 8 min and tailed to as long as 20 min. Fractions were checked for heroin using the method described for morphine, using GC-FID or GC/MSD (SIM set to heroin's base peak mass; $m/z = 327$); again, all heroin fractions were combined. The resulting combined solution was made basic with solid sodium bicarbonate to pH 8, then extracted 5 times with equal amounts of chloroform. The chloroform extracts were combined, dried with sodium sulfate, filtered, and evaporated off using a rotevap and a high vacuum pump. Purities of 95 to 99% were normal; these were determined using the area percent of peaks using GC-FID. For those cases where purity was less than the minimum target value of 95%, purity was raised to 95% or above using ethyl acetate or acetone to recrystallize the heroin base.

NMR Analysis

Samples and internal standard (nitromethane for seized heroin samples and methenamine for opium-heroin and cocaine samples) were accurately weighed into a test tube. Hexafluorobenzene (for fluorine lock) and protonated chloroform were added to sample tube, mixed, filtered, and transferred to a 10 mm NMR sample tube. The deuterium spectra were recorded at 308K using a 10 mm outside diameter (OD) deuterium probe, broadband decoupling and 19F locking (provided by the hexafluorobenzene). The quantitative measuring conditions were as follows: 90° pulse (13 μs), repetition delay of 5 times T1max (2 s), sweep width 4800 Hz, time-domain size of 32K, acquisition time of 2 s. The chemical shifts are expressed in ppm with respect to TMS.

After feasibility studies indicated insufficient sample sizes, the 19 preliminary seized heroin samples were combined into four "regional samples" and analyzed three times for 12 h each as a repeatability study. The 17 opium-heroin samples were analyzed for 20 h; four of these analyzed twice at 10 h apiece. The internal standard was changed from nitromethane to methenamine for the opium-heroin samples in the hopes of reducing spectrometer times; in addition, nitromethane slowly reacted with the heroin (indicated by discoloration of the NMR solution), and therefore reduced the usefulness of the samples for future analysis.

Data from the 17 opium-heroin samples were examined by principal component analysis (PCA), discriminant function analysis (Table 1), and variance analysis (ANOVA). F-Test and p-Test values for individual signals in the spectrum were also calculated (Table 2).

TABLE 1—*Discriminant function analysis for opium-heroin samples; percent assignment of samples in groups.*

Country	% Correct	India	Korea	Turkey
India	80	8	0	2
Korea	100	0	7	0
Turkey	75	2	0	6
Total	84	10	7	8

TABLE 2—*F-Test and p-Test results for 16 opium-heroin samples.*

Peak(s)	F-Test	p-level (%)
1 + 2	5.23	1.4
7 + 8	4.82	1.8
15a	2.91	7.6
10a	2.34	12.0
16a	1.56	23.3
15b	1.05	36.5
14	0.89	42.4
NCH_3	0.83	45.1
5 + 6	0.27	76.9
16b	0.24	78.6
9	0.14	86.8

Results and Discussion

Preliminary Study on Seized Heroin Exhibits

The initial study used a 100 mg sample, and was analyzed for 70 h. This sample size was insufficient to obtain an appropriate signal-to-noise ratio, due to the relatively high molecular weight of heroin. Therefore, all samples from each of the four regions were combined into "regional samples" and run to improve the signal-to-noise and reduce analysis time. The combined heroin samples were from the four following regions: South America (5 samples; totaling 0.70 g), Southeast Asia (4 samples; totaling 0.46 g), Southwest Asia (4 samples; totaling 0.67 g) and Mexico (6 samples; totaling 0.64 g). These four "regional samples" were run for 12 h each, and repeated three times each, giving the spectrum in Fig. 3 and the results presented in Tables 3 and 4.

The quantitative analysis of the spectra is based on a global least-squares curve fitting approach (43). This algorithm, which has been developed by Eurofins Laboratories, proved to be very precise. For instance, in the preliminary work containing three measurements on each sample, the percent relative standard deviation (%RSD) of the molar fraction of heroin peak 16b, a peak completely hidden in a cluster of other peaks, was less than 3%. Table 4 gives the normalized molar fractions (molar fraction divided by the average molar fraction for the site), variance, and discrimination value for the preliminary heroin study. A high discrimination value indicates that a specific site may have significance in differentiating one source of heroin from another. The molar fractions of clusters of peaks 1 + 2 and 7 + 8 are more accurate than the molar fraction of the individual peaks, a well known phenomenon of highly overlapped peaks. The exact deuterium ratio (D/H) was not determined for either study because the internal standards were not calibrated. This was not necessary for this project, since the study was only concerned with the ability of the method to discern country of origin, not in the actual deuterium amounts at the various sites.

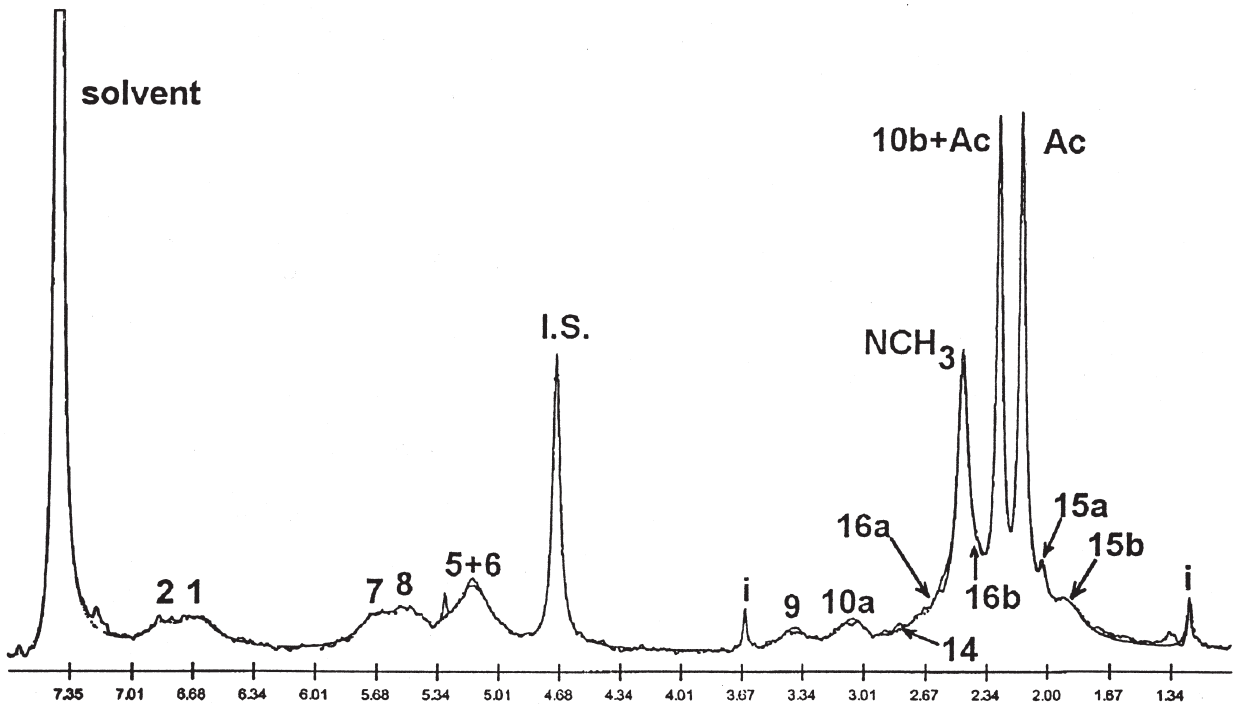

FIG. 3—*Deuterium spectrum of heroin base in chloroform (solvent) using nitromethane as internal standard (I.S.). Assignments correspond to positions on heroin as shown in Fig. 1*a. *The two "Ac" peaks are the synthetic acetyl groups and the two "i" peaks are impurities.*

TABLE 3—*Normalized molar fractions (molar fraction divded by all molar fractions in the spectrum) for heroin base. Three spectra per sample obtained to determine precision of method. Values for standard deviation (SD), and percent relative standard deviation (%RSD = SD/mean × 100) presented. Samples are from the regions of Southwest Asia (SWA), Southeast Asia (SEA), South America (SA), and Mexico (Mex). See Fig. 1*a *for deuterium site numbers.*

Site No.	SWA 1	SWA 2	SWA 3	SEA 1	SEA 2	SEA 3	SA 1	SA 2	SA 3	Mex 1	Mex 2	Mex 3	SD	%RSD
2	0.0303	0.0309	0.0160	0.0292	0.0274	0.0304	0.0274	0.0352	0.0303	0.0334	0.0263	0.0340	0.0052	17.7000
1	0.0422	0.0426	0.0539	0.0444	0.0411	0.0385	0.0470	0.0480	0.0458	0.0436	0.0536	0.0421	0.0048	10.7000
7	0.0336	0.0340	0.0273	0.0420	0.0382	0.0405	0.0388	0.0442	0.0418	0.0421	0.0403	0.0427	0.0026	6.6000
8	0.0516	0.0519	0.0613	0.0500	0.0514	0.0512	0.0507	0.0414	0.0511	0.0538	0.0544	0.0555	0.0039	7.6000
5 + 6	0.1066	0.1069	0.1080	0.1035	0.1004	0.0941	0.1110	0.1168	0.1135	0.1082	0.1098	0.1058	0.0030	2.8000
9	0.0291	0.0301	0.0327	0.0284	0.0199	0.0224	0.0250	0.0215	0.0283	0.0292	0.0268	0.0250	0.0031	11.7000
10a	0.0400	0.0407	0.0451	0.0368	0.0343	0.0332	0.0382	0.0363	0.0405	0.0451	0.0382	0.0392	0.0027	7.0000
14	0.0103	0.0113	0.0144	0.0032	0.0024	0.0056	0.0033	0.0009	0.0119	0.0099	0.0036	0.0096	0.0037	50.8000
16a	0.0660	0.0677	0.0726	0.0722	0.0634	0.0623	0.0579	0.0618	0.0648	0.0732	0.0666	0.0659	0.0042	6.3000
NCH_3	0.1518	0.1513	0.1479	0.1453	0.1526	0.1483	0.1558	0.1498	0.1563	0.1598	0.1579	0.1613	0.0029	1.9000
16b	0.0694	0.0699	0.7130	0.0789	0.0720	0.0767	0.0793	0.0802	0.0768	0.0778	0.0747	0.0755	0.0022	2.9000
10b + Ac	0.1412	0.1405	0.1423	0.1428	0.1503	0.1497	0.1233	0.1199	0.1195	0.1255	0.1273	0.1285	0.0025	1.9000
Ac	0.1604	0.1584	0.1548	0.1647	0.1722	0.1722	0.1576	0.1614	0.1532	0.1493	0.1557	0.1535	0.0037	2.3000
15a	0.0299	0.0277	0.0189	0.0146	0.0259	0.0244	0.0324	0.0299	0.0199	0.0128	0.0230	0.0210	0.0060	25.8000
15b	0.0377	0.0361	0.0333	0.0440	0.0485	0.0505	0.0522	0.0527	0.0462	0.0362	0.0418	0.0403	0.0031	7.0000
1 + 2	0.0725	0.0736	0.0699	0.0737	0.0685	0.0689	0.0744	0.0832	0.0762	0.0770	0.0799	0.0761	0.0031	4.1000
7 + 8	0.0852	0.0859	0.0887	0.0920	0.0895	0.0917	0.0895	0.0856	0.0929	0.0960	0.0947	0.0982	0.0023	2.6000
mg sample	0.520	0.520	0.520	0.610	0.610	0.610	0.521	0.521	0.521	0.579	0.579	0.579		
mg I.S.	0.340	0.340	0.340	0.342	0.342	0.342	0.342	0.342	0.342	0.337	0.337	0.337		

TABLE 4—*Normalized molar fractions (molar fraction divided by the average molar fraction for the site) for heroin base in descending discrimination order. Samples are from the regions of Southwest Asia (SWA), Southeast Asia (SEA), South America (SA), and Mexico (Mex). See Fig. 1*a *for deuterium site numbers.*

Site No.	SWA	SEA	SA	Mex	1 − SD	1 + SD	Discrimination
AcO	1.05	1.10	0.90	0.95	0.98	1.02	4.9376
15b	0.82	1.10	1.16	0.91	0.93	1.07	2.2550
5 + 6	1.00	0.93	1.06	1.01	0.97	1.03	1.9748
AcO	0.99	1.06	0.99	0.96	0.98	1.02	1.9512
7 + 8	0.95	1.00	0.98	1.06	0.97	1.03	1.7506
NCH_3	0.98	0.97	1.01	1.04	0.98	1.02	1.6734
16b	0.93	1.01	1.05	1.01	0.97	1.03	1.6525
1 + 2	0.97	0.94	1.05	1.04	0.96	1.04	1.2677
10a	1.08	0.89	0.98	1.05	0.93	1.07	1.1691
9	1.16	0.89	0.94	1.02	0.88	1.12	0.9980
14	1.66	0.52	0.75	1.07	0.49	1.51	0.9785
16a	1.04	1.00	0.93	1.04	0.94	1.06	0.8100
15a	1.09	0.93	1.17	0.81	0.74	1.26	0.6321

Heroins from 17 Opium Samples of Known Origin

The preliminary DNMR study indicated that 1 g of heroin base was actually needed for each experiment; however, no authentic heroin samples were available in those amounts. Therefore, authentic opium samples from Turkey (6 samples), Korea (5 samples) and India (6 samples) were taken and processed to morphine and then heroin. Because preliminary work using a typical clandestine method[8] for isolating the morphine from the other opium alkaloids gave poor results (and this method also would not have adequately separated morphine and codeine), reverse phase preparative HPLC was employed, as described earlier, to isolate morphine.

Determination of the Geographical Origin of Heroin (Without Any Information on the Origin of the Products)

In order to visualize potential geographical dependencies of analytical parameters, a principal component analysis of the isotopic molar fractions reported in Tables 5 and 6 was conducted. The duplicate measurement of samples 26, 30, 31, and 36 are clearly visualized in Figs. 4–6. The 95% confidence ellipses are displayed for each group in these figures.

The F-Test and p-Test results are found in Table 2. The F-Test is the result of the ANOVA variance analysis and corresponds basically to the distances between the groups divided by the standard deviation of each population. The p-Test corresponds to the same results expressed as a level of confidence. The smaller the p-Test is, the better the discrimination.

Determination of the Geographical Origin of Heroin (With Some Information on the Origin of the Products)

Discriminate function analysis takes the geographical origin into account and tries to maximize the differences between the groups (Fig. 7). To give an idea of the significance of the differences observed between the groups, this graph can be compared with a graph resulting from the same statistical analysis but based on random numbers instead of real results (Fig. 8). The groups obtained in the simulation have obviously more overlapping than for the isotopic profile of heroin.

The results confirm that there is a significant geographical effect on the isotopic parameters between the groups. However, this effect is not large enough to determine the geographical origin of individual samples, because the overlaps between groups remain significant. In other words, the isotopic profile of heroin gives some information on the geographical origin of the heroin, but not enough to be successfully used alone as a method to determine the country of origin.

Variances With the Acetyl Group Deuteriums

The acetyl group measurements for the 17 opium-heroin samples (Table 5) should have been the same, within experimental error, since all morphine samples were subjected to the same acetic anhydride and the same conditions for acetylation. In fact, they are not, and instead show great variation. This means that the reaction on a small gram scale generates fractionation. This should not be the case for samples produced in kilogram quantities. The variance of the 17 opium-heroin sample acetyls was quite large (16.8 and

[8] The opium is added to hot water, then made basic (pH $>$ 10) with calcium hydroxide (to complex the morphine with calcium), then filtered and brought to pH 8 with ammonium chloride; morphine base precipitates. However, in practice, crystals did not always form and yields were very poor.

TABLE 5—*Isotopic molar fractions of heroin produced from opium relative to the internal standard methenamine. See Fig. 1a for deuterium site locations.*

Site No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
1 + 2	0.0399	0.0865	0.0518	0.0395	0.0249	0.0312	0.0422	0.0582	0.0903	0.0501	0.0528	0.0448	0.0475	0.0463	0.0358	0.0427	0.0539
7 + 8	0.0571	0.0425	0.0163	0.0436	0.0382	0.0569	0.0484	0.0260	0.0400	0.0663	0.0618	0.0528	0.0417	0.0376	0.0327	0.0301	0.0402
5 + 6	0.0513	0.0482	0.0500	0.0722	0.0391	0.0500	0.0479	0.0395	0.0622	0.0555	0.0836	0.0571	0.0772	0.0714	0.0476	0.0710	0.0732
9	0.0096	0.0122	0.0149	0.0113	0.0078	0.0129	0.0142	0.0059	0.0090	0.0120	0.0146	0.0136	0.0131	0.0115	0.0101	0.0120	0.0141
10a	0.0178	0.0182	0.0111	0.0191	0.0135	0.0214	0.0188	0.0096	0.0149	0.0178	0.0235	0.0218	0.0190	0.0193	0.0170	0.0192	0.0219
14	0.0170	0.0220	0.0028	0.0196	0.0144	0.0223	0.0215	0.0089	0.0138	0.0193	0.0223	0.0222	0.0191	0.0172	0.0093	0.0224	0.0086
16a	0.0168	0.0174	0.0313	0.0187	0.0113	0.0189	0.0181	0.0131	0.0203	0.0164	0.0234	0.0191	0.0202	0.0197	0.0350	0.0216	0.0373
NCH_3	0.0757	0.0830	0.0742	0.0865	0.0590	0.0833	0.0788	0.0373	0.0579	0.0881	0.1037	0.0894	0.0810	0.0785	0.0634	0.0788	0.0909
16b	0.0114	0.0124	0.0091	0.0140	0.0085	0.0124	0.0125	0.0104	0.0162	0.0124	0.0170	0.0141	0.0145	0.0150	0.0096	0.0138	0.0155
AcO	0.0816	0.0829	0.0798	0.0849	0.0574	0.0846	0.0763	0.0387	0.0601	0.0966	0.1050	0.0918	0.0921	0.0915	0.0746	0.0855	0.1009
AcO	0.0860	0.0905	0.0844	0.0883	0.0613	0.0862	0.0793	0.0496	0.0768	0.1004	0.1078	0.0918	0.0939	0.0915	0.0762	0.0862	0.1035
15a	0.0266	0.0283	0.0176	0.0138	0.0137	0.0108	0.0157	0.0171	0.0265	0.0187	0.0178	0.0145	0.0175	0.0161	0.0115	0.0129	0.0197
15b	0.0191	0.0199	0.0358	0.0184	0.0123	0.0108	0.0156	0.0200	0.0311	0.0283	0.0291	0.0230	0.0253	0.0269	0.0189	0.0238	0.0269
Country	Turkey	Turkey	Korea	Korea	India	India	Turkey	India	Korea	Turkey	India	India	Turkey	Turkey	Korea	Korea	Turkey
mg sample	0.9118	0.9232	0.3580	0.9103	0.9210	0.9143	0.9236	0.9041	0.4910	0.9627	0.8698	0.9352	0.9254	0.9190	0.7001	0.9090	0.9096
mg I.S.	0.0561	0.0509	0.0519	0.0693	0.0595	0.0539	0.0505	0.0594	0.0501	0.0606	0.0645	0.0514	0.0673	0.0634	0.0567	0.0727	0.0708

TABLE 6—*Isotopic molar fractions of heroin produced from opium relative to the internal standard methenamine. Samples that were repeated twice to verify precision.*

Site No.	26A	26B	30A	30B	31A	31B	36A	36B
1 + 2	0.0949	0.0779	0.0289	0.0336	0.0403	0.0441	0.0441	0.0456
7 + 8	0.0308	0.0546	0.0602	0.0536	0.0453	0.0515	0.0508	0.0548
5 + 6	0.0540	0.0422	0.0479	0.0521	0.0476	0.0482	0.0607	0.0534
9	0.0114	0.0131	0.0128	0.0130	0.0145	0.0139	0.0137	0.0135
10a	0.0184	0.0181	0.0213	0.0215	0.0180	0.0196	0.0209	0.0228
14	0.0215	0.0224	0.0229	0.0217	0.0213	0.0217	0.0214	0.0231
16a	0.0175	0.0174	0.0193	0.0185	0.0179	0.0183	0.0170	0.0212
NCH_3	0.0797	0.0864	0.0824	0.0842	0.0765	0.0811	0.0895	0.0892
16b	0.0125	0.0124	0.0117	0.0132	0.0131	0.0119	0.0140	0.0143
AcO	0.0815	0.0843	0.0840	0.0852	0.0753	0.0772	0.0900	0.0937
AcO	0.0895	0.0916	0.0847	0.0877	0.0774	0.0811	0.0909	0.0927
15a	0.0285	0.0282	0.0111	0.0105	0.0154	0.0160	0.0142	0.0149
15b	0.00207	0.0191	0.0097	0.0118	0.0137	0.0174	0.0209	0.0251

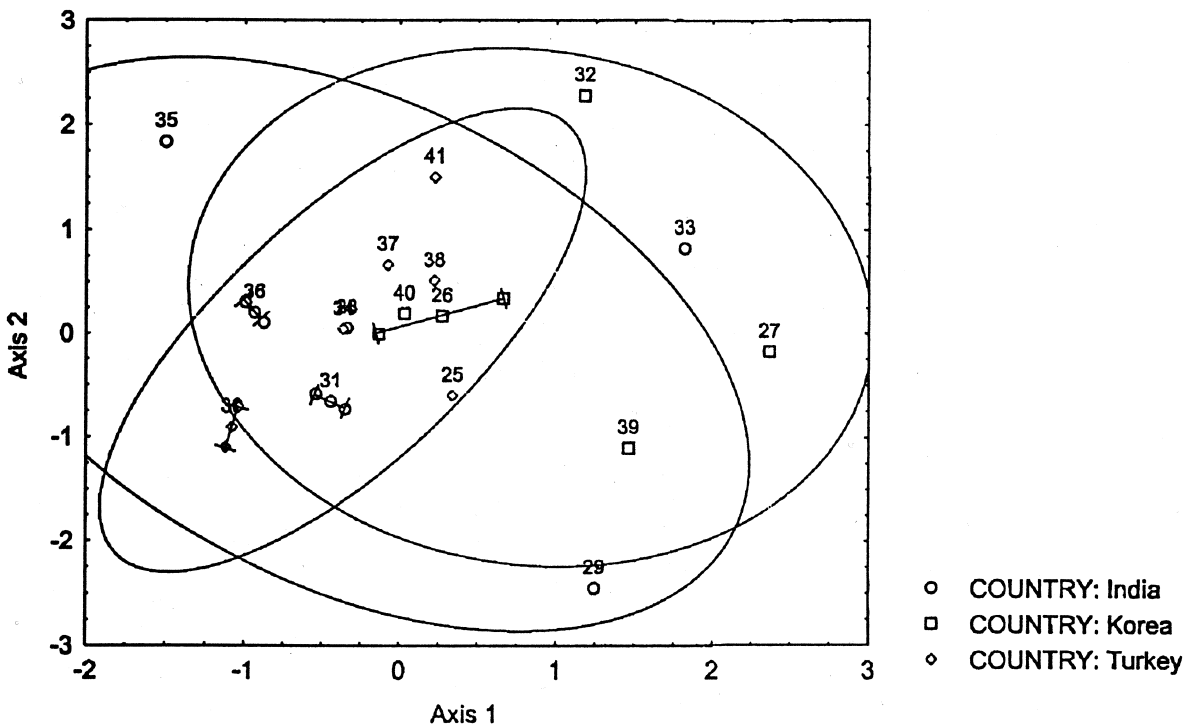

FIG. 4—*Principal component analysis (Axis 1 and Axis 2).*

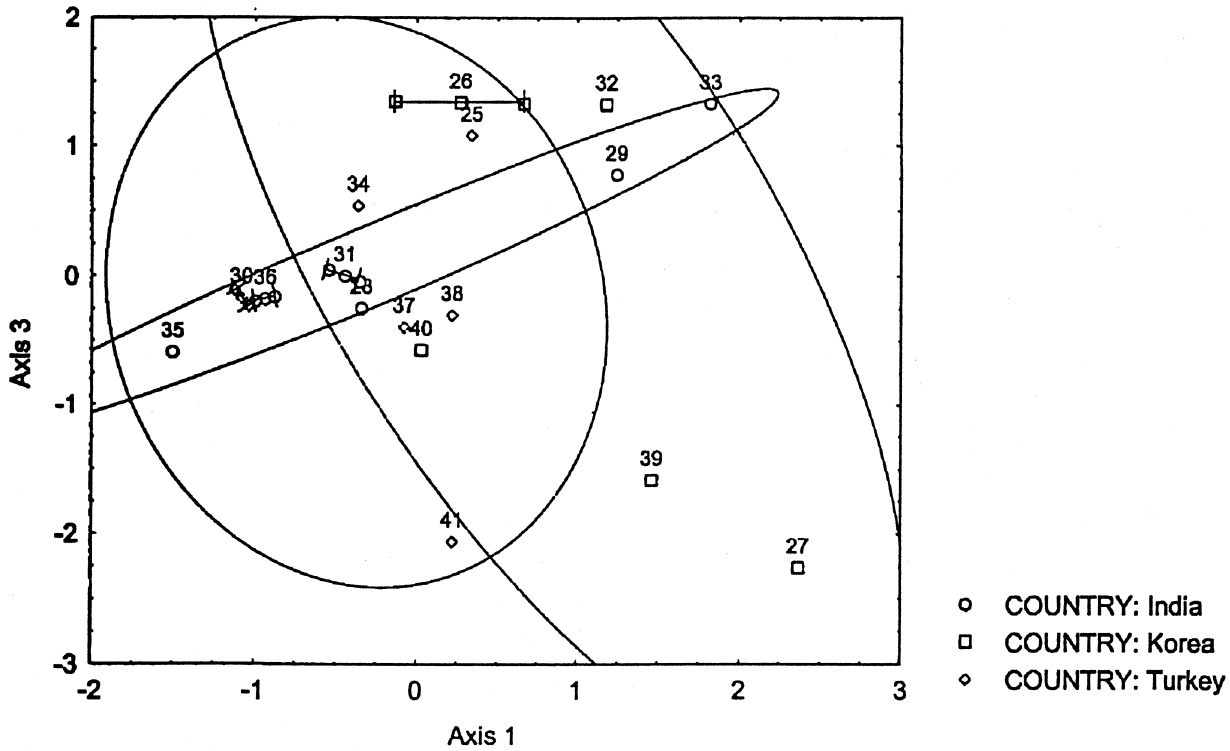

FIG. 5—*Principal component analysis (Axis 1 and Axis 3).*

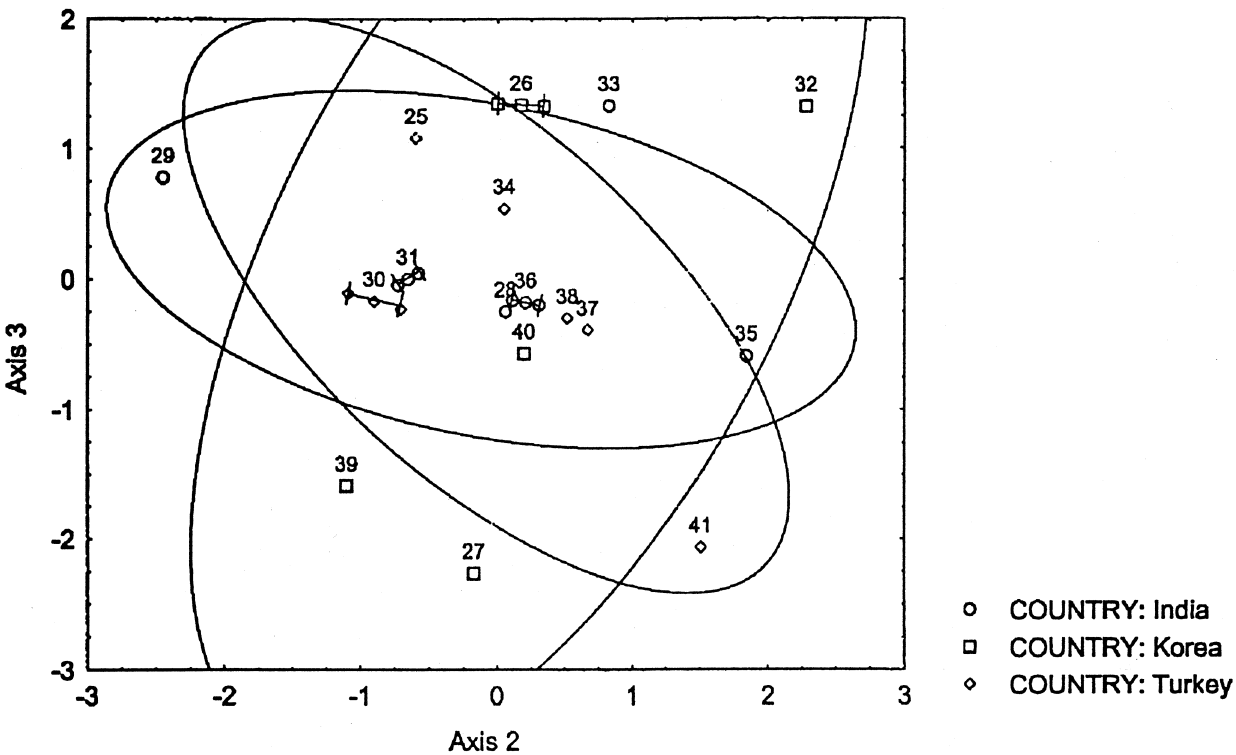

FIG. 6—*Principal component analysis (Axis 2 and Axis 3).*

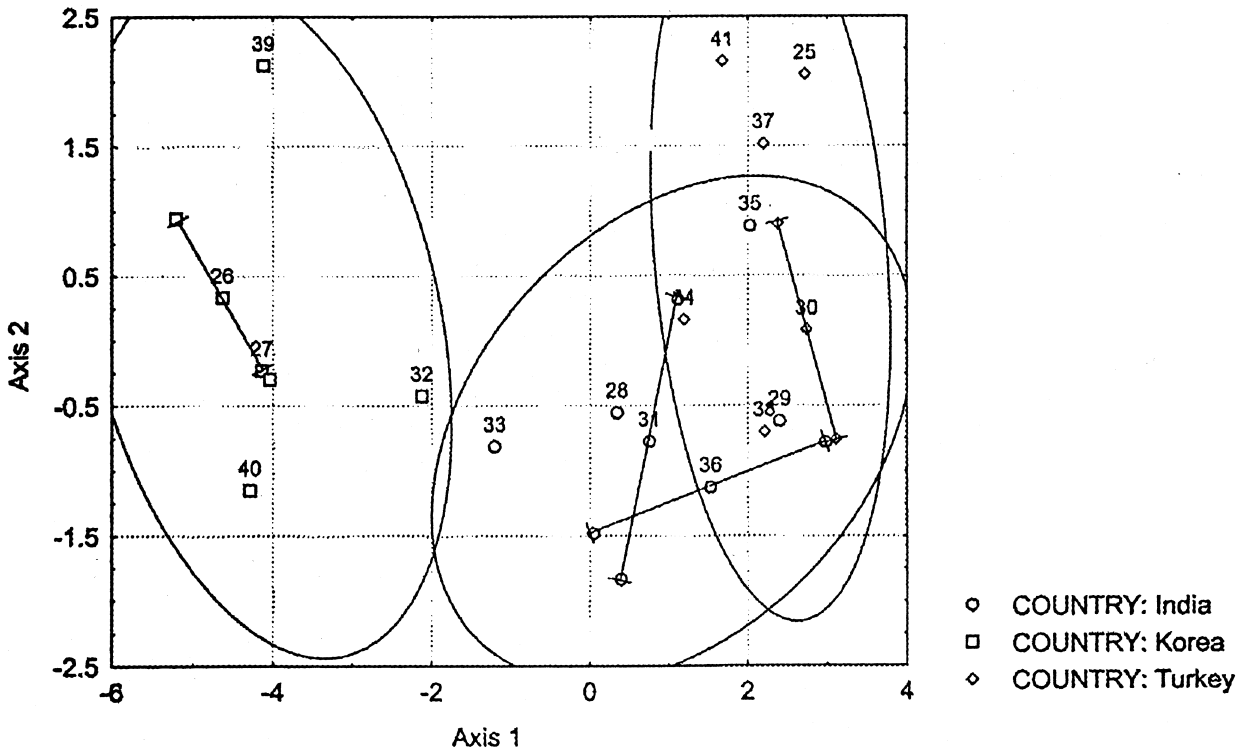

FIG. 7—*Discriminant analysis.*

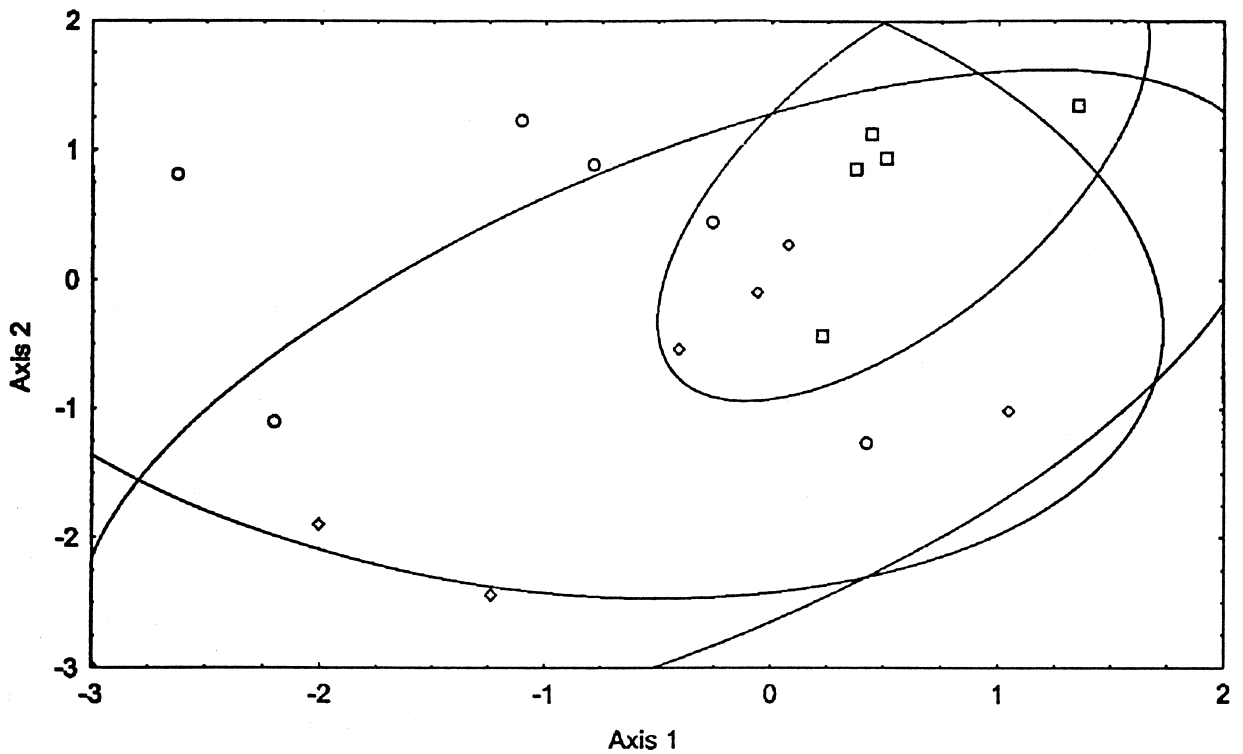

FIG. 8—*Simulation. India = ○, Korea = □, Turkey = ◊.*

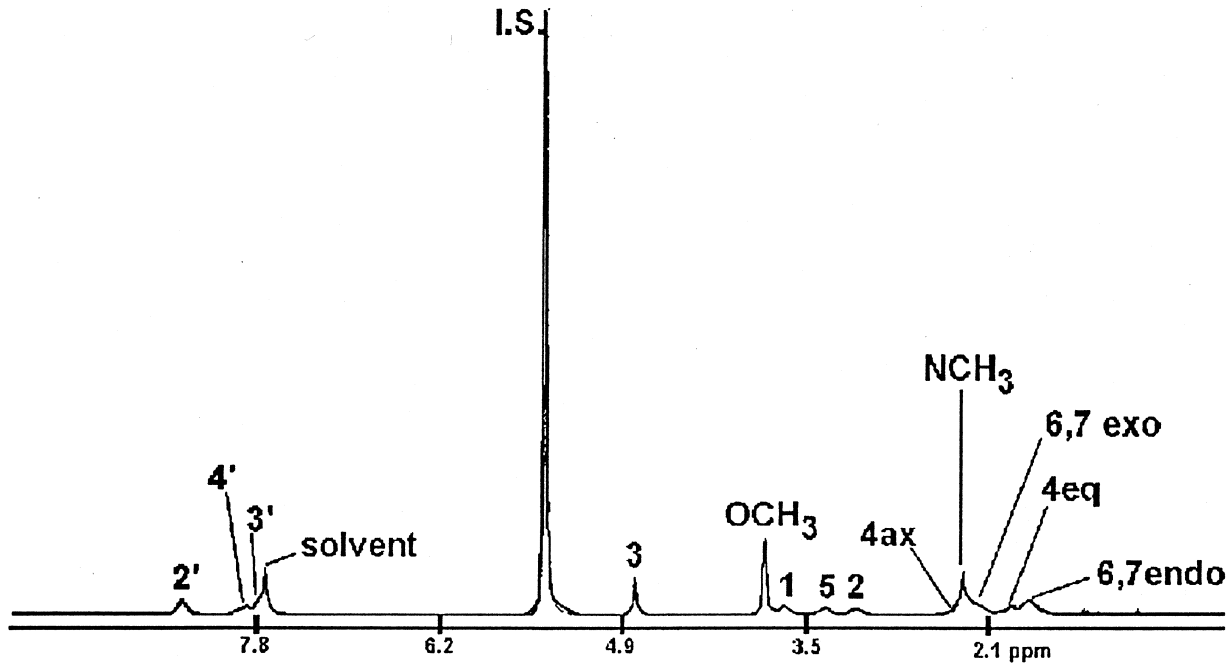

FIG. 9—*Deuterium spectrum of cocaine base in chloroform (solvent) using methenamine as the internal standard (I.S.).*

TABLE 7—*Isotopic molar fractions of cocaine base relative to the internal standard methenamine. See Fig. 1*b *for cocaine site numbers.*

Site No.	Peru	Bolivia
2′	0.0831	0.1022
4′	0.0467	0.0253
3′	0.0899	0.0947
3	0.0862	0.0934
$COOCH_3$	0.1331	0.1573
1	0.0648	0.0582
5	0.0304	0.0389
2	0.0347	0.0365
4 axial	0.0106	0.0169
NCH_3	0.1275	0.1536
6 exo + 7 exo	0.1418	0.1476
4 equitorial	0.0247	0.0321
6 endo + 7 endo	0.1265	0.1402

20.5% RSD), while the acetyl data from the earlier study on heroin from the four major producing regions was quite a bit smaller (1.1% RSD among all samples).

Preliminary Study of Cocaine Base of Known Origin

Two 1 g samples of cocaine base of known origin (Peru and Bolivia) were also analyzed as a preliminary study to see how well DNMR works with this compound. In this case, the objectives were: (*a*) to determine the number of quantitative deuterium sites; and (*b*) to determine the differences in the deuterium abundances between the two samples. The same instrument parameters were used for these samples as for heroin. The spectrum (Fig. 9) shows good resolution of most of the deuterium signals, and the results presented in Table 7 show substantial differences in isotopic molar ratios for the deuterium sites. While this is promising, further work must be undertaken with a much larger database of samples of known origin before any significant conclusions can be drawn.

Conclusions

Based on this study, and this sample set, there is a geographical effect on the isotopic parameters of heroin, and deuterium NMR can give some useful information for determining the geographical origin of heroin when associated with other analytical techniques (IRMS, impurity profiling, etc.).

The case of heroin is unusually difficult because of the size of the molecule and the numerous processing methods of opium to heroin. One can reasonably expect to get significantly better results using the deuterium NMR approach with smaller, naturally occurring molecules (e.g., cocaine and tetrahydrocannabinol) to determine geographic origin, or with synthetic drugs (e.g., methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, etc.) to identify precursor origin.

Note, however, that this study used the same method for processing all opium samples into heroin. This is not the case in clandestine settings. There is great variability between methods throughout the world, and even between clandestine chemists within the same region. This variability could have adverse effects on the deuterium concentrations at certain sites due to inadvertent isotopic fractionation or chemical reactions which create "labile" sites. Further study is needed with actual samples from known origin.

In addition, the probe which was utilized in this study is ideal for liquids and samples of large quantity, but may not be the best for analysis of small and large molecules where sample availability is limited. There have been recent, major advances in probe technology that could make it possible to perform DNMR on compounds with molecular weights of 400 or more using 100 mg of sample (and perhaps less). Also, the use of higher field magnets would also increase sensitivity and improve peak resolution.

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