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TECHNICAL NOTE

CRIMINALISTICS

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Comparison of Bulk and Compound-Specific $\delta^{13}C$ Isotope Ratio Analyses for the Discrimination Between Cannabis Samples*

ABSTRACT: Five marijuana samples were compared using bulk isotope analysis compound-specific isotope ratio analysis of the extracted cannabinoids. Owing to the age of our cannabis samples, four of the five samples were compared using the isotope ratios of cannabinol (CBN), a stable degradation product of Δ^9-tetrahydrocannabinol (THC). Bulk $\delta^{13}C$ isotope analysis discriminated between all five samples at the 95% confidence level. Compound-specific $\delta^{13}C$ isotope analysis could not distinguish between one pair of the five samples at the 95% confidence level. All the measured cannabinoids showed significant depletion in ^{13}C relative to bulk isotope values; the isotope ratios for THC, CBN, and cannabidiol were on average 1.6‰, 1.7‰, and 2.2‰ more negative than the bulk values, respectively. A more detailed investigation needs to be conducted to assess the degree fractionation between the different cannabinoids, especially after aging.

KEYWORDS: forensic science, marijuana, cannabinoids, cannabinol, cannabidiol, isotope ratio mass spectrometry, compound-specific isotope ratios, bulk isotope ratios, gas chromatography, mass spectrometry, carbon isotope ratio, $\delta^{13}C$, drugs of abuse

According to the World Drug Report 2008, marijuana or *Cannabis sativa* L. is the most frequently abused illicit drug in the world (1). One hundred sixty-six million people, or *c*. 3.9% of the world population, are estimated to abuse the drug (1). In 2006, 10% of the 8th graders, 25% of the 10th graders, and 32% of the 12th graders in the United States abused marijuana (2). Some teenagers justify their marijuana consumption by claiming relief from different health problems, such as depression, sleeping difficulties, and physical pain (3). It is reported in a variety of different publications that adolescents tend to have difficulties in concentration, attention, and learning after repeated drug intake (4).

The main active constituents in *C. sativa* L. are cannabinoids such as cannabinol (CBN), cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC), cannabigerol, and cannabichrome (5). Their structures are shown in Fig. 1. *C. sativa* L. also contains different terpenes (e.g., mycrene), sesquiterpenes (e.g., Caryophyllene), and terpenoid-like compounds (6), although these are not thought to be psycoactive. More than 60 cannabinoids have now been identified in *C. sativa* L.; however, the major psychoactive effects are attributable to THC (7). THC content varies for different plant parts in the following order: bracts > flowers > leaves > smaller stems > larger stems > roots > seeds (8). The average THC content in seized samples ranges from 1 to 14%. For Sinsemilla, or plants cultured in the Netherlands, Switzerland, and British Columbia, the THC content can be up to 20%. Cannabinoids can be detected in the bodily fluids of excessive users up to several weeks after stopping the intake of THC. This is owing to the accumulation of THC in fatty tissue facilitated by its lipophilic properties (9). Jung et al. (10) showed the detection of 9-tetrahydrocannabinolic acid and THC in human blood serum and urine using liquid chromatography–tandem mass spectrometry (LC/MS/MS). THC can also be detected in fingernails (11) and hair (12). Recent drug use can be detected in the saliva of suspected users through the use of liquid/liquid extractions and gas chromatography–mass spectrometry (GC/MS) (13).

In criminal cases, the forensic analysis of marijuana-related cases typically involves the confirmation of drug identity for the prosecution of crimes such as possession, possession with intent to distribute, and trafficking. Microscopic and macroscopic botanical features and the Duquenois–Levine color test are most commonly used to identify exhibits as originating from *C. sativa*, and GC/MS is used to confirm the presence of THC when botanical features are not present. In civil cases, the identification of drug and drug metabolites in samples from suspected users are commonly used to establish drug use and abuse. Federal agencies are often interested in the drugs' origin and in determining large-scale trafficking routes for potential legal/enforcement solutions. GC/MS of cannabis extracts with various statistical treatments has also been used with success to discriminate between cannabis plants of different strains or different geographic origins (6,14,15).

C. sativa L. is a C_3 plant-like corn, cotton, and soy (16), and its photosynthesis activities can be recorded in the isotope ratio of carbon (17). Environmental factors such as climate, water availability, temperature, and light intensity also have an impact on the assimilation of ^{13}C. By approximation, the background $\delta^{13}C$ value for atmospheric CO_2 is -8 ± 0.2‰ (18). Plant tissues are depleted with respect to the atmospheric source because photosynthesis and enzymatic fixation are discriminating against the heavier isotope. *C. sativa* L. follows the Benson–Calvin (C_3) photosynthetic pathway and therefore shows $\delta^{13}C$ values typically in the range of −24‰ to −35‰, depending upon the growth conditions (19). The ratio of the CO_2 concentration in the stomata to the concentration

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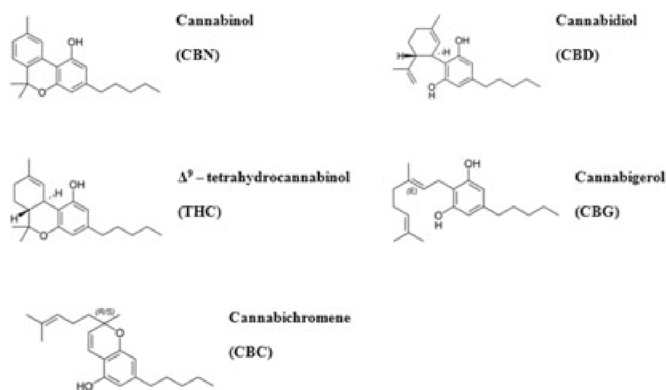

FIG. 1—*Cannabinoid structures of the main active constituents in* Cannabis sativa *L.*

in the air is controlled by the stomatal conductance relative to the photosynthetic activity. Interpretation of this relationship indicates the growth conditions (indoor or outdoor growth) (20–23). Because of the various contributing factors to the ^{13}C content of cannabis plants, isotope ratio mass spectrometry (IRMS) of carbon, and other light elements can therefore be used as a forensic tool for establishing the common origin of seizures and, with many caveats, in tracking the geographic origin of seized samples (22,24).

Depending on the nature of the sample, there are two common methods to introduce the sample into the IRMS system: an elemental analyzer (EA) for solid or bulk samples and a GC for complex mixtures. EAs require the combustion of a solid or liquid sample and provide gross or bulk isotope ratio values of the entire physical sample. The advantage of the GC, as compared to the EA, is that there is a pre-separation of the individual components before isotopic analysis. Therefore, the isotope ratios measured after chromatographic separation are compound-specific and can provide significantly more detail about a complex sample than can a single weighted average (bulk) value. To date, several researchers have used EA/IRMS to provide bulk analyses of cannabis samples (19,20,22–26). However, to the best of our knowledge, no results using GC/IRMS of individual cannabinoids can be found in the extant literature. Here, we use a GC to separate the cannabinoid extracts of *C. sativa* L. plant material and simultaneous electron ionization (EI)/MS and combustion (C)/IRMS detectors to identify and measure the isotope ratios of each separated component, respectively. Subsampling and analysis of variance (ANOVA) were used to establish the sources of variance in the analyses. The sampling variance was found to be significantly smaller than the measurement variance in all cases, thereby indicating that the samples were relatively homogeneous (in terms of isotopic composition) and that the power of discrimination between samples could be improved through improving (i.e., reducing) the measurement error.

Materials and Methods

Chemicals

The samples of marijuana utilized in our research comprised four aged street samples obtained through local law enforcement agencies and one fresh sample grown in-house by a local enforcement agency (Bureau of Criminal Identification and Investigation [BCI] Lab in London, Ohio). The original source of four aged street samples in our inventory is not known. Four of the samples were more than 10 years old and although the samples were known to originally contain significant amounts of THC, the THC has now decomposed into CBD and CBN (27). The cannabinoids CBN and CBD were shown to be dominant components in these four samples and are still specific to cannabis plants. The newly obtained cannabis plant matter contained large amounts of THC relative to the other cannabinoids, but because this sample had not undergone aging, it did not contain useful quantities of CBN and CBD.

Samples were re-labeled A–E for this study. Original sample sizes for samples A–D varied from *c.* 1 to 20 g. Sample E, the newly grown sample provided by BCI was *c.* 35 g of dried leaf matter. Different subsamples (aliquots) were taken at random locations of each sample for subsequent analysis. Approximately 300 mg of leaf matter from each aliquot was pulverized by adding six to eight stainless steel ball bearings and rapidly shaking the vial for 5 min using a Mini Beadbeater (Biospec Products Inc., Bartlesville, OK). Approximately 200 mg of each powdered marijuana sample was then put in a 1.5-mL glass vial and extracted with 1 mL of acetone (Sigma Aldrich, St. Louis, MO). The subsamples were then sonicated for 15 min before centrifuging for 2 min to pelletize the solid plant matter. The liquid supernatant was then filtered to remove any particulates. One hundred and fifty microliters of the remaining subsample was then transferred to a 1.5-mL autosampler vial with 200 μL microwell inserts and placed into the autosampler carousel to await injection.

Bulk Isotope Ratio Mass Spectrometry

Bulk carbon isotope measurements were made on precisely weighed samples of *c.* 1 mg that were placed in tin capsules in a Costech ECS 4010 EA (Costech Analytical Technologies, Inc., Valencia, CA). The EA was coupled via a Conflo III interface (Thermo Finnigan, Waltham, MA) to the Thermo Delta Plus Advantage. Data acquisition was carried out using Isodat 2.0 Software (Thermo Finnigan). During both the GC and EA analyses, high-purity gases from Airgas (Parkersburg, WV) were used: >99.9999% He, 99.999% N_2, 99.997% CO_2, and 99.999% O_2. The reference cylinder of CO_2 gas was calibrated using replicate analyses of USGS standards USGS40 and USGS41. For quality control, an IAEA isotope standard of caffeine (IAEA-600, $\delta^{13}C$ = −27.77‰) was run on both the EA-IRMS and GC-C-IRMS instruments and provided values of −27.74‰ ($N = 4$) and −27.75‰ ($N = 6$), respectively, thus validating the accuracy of the two measurement systems.

Gas Chromatography–Mass Spectrometry/Isotope Ratio Mass Spectrometry

Separations were performed on a gas chromatograph (Trace GC; Thermo Finnigan) equipped with an autosampler (AS3000; Thermo Finnigan). Detection was accomplished using dual mass spectrometer detectors, as described previously (28). Approximately 10% of the column effluent was split to a single-quadrupole mass spectrometer (HP5970B; Hewlett Packard [now Agilent], Santa Clara, CA) for EI fragmentation analysis, and the remaining 90% was split to an isotope ratio mass spectrometer (Delta Plus Advantage; Thermo Finnigan) through the GC Combipal III interface for isotopic analysis. The GC column was a 5% Phenylpolydimethylsiloxane (DB-5) 60 m × 0.25 mm × 0.25 μm column (J & W Scientific, Folsom, CA). The GC effluent was split using a low-dead-volume X-connector (Valco Instruments Co., Inc., Houston, TX).

The GC oven was held at 100°C for 5.0 min and then ramped at 20°C/min to a final temperature 300°C for 8.0 min. The total separation time was 23 min. The injector temperature was set at 280°C, and the helium carrier gas flow rate was 2.5 mL/min. A volume of

1 μL was injected in splitless mode using the aforementioned autosampler. The temperature of the auxiliary transfer line to the single quadrupole was set at 300°C.

EI mass spectra were recorded between the ranges of *m/z* 50–550 using HP Chemstation B.02.05 (Hewlett Packard [now Agilent]). Postacquisition, the data were exported as a text file for comparison with the NIST mass spectral library (U.S. Secretary of Commerce) to confirm identify of the cannabinoids of interest. For the IRMS measurements, the combustion oven temperature was held at 940°C, and the reduction oven temperature was held at 650°C. Data acquisition was carried out using Isodat 2.0 Software (Thermo Finnigan).

We began each run with three injections of the high-purity (99.997%) CO_2 reference gas (Airgas; Great Lakes, Independence, OH), of which the second CO_2 peak is designated as the standard to be used for calculating the $\delta^{13}C$ values by the instrument acquisition software. The isotope standard and samples were injected, separated, and analyzed under identical conditions, thereby adhering as closely as possible to the identical treatment principle (29) for IRMS calculations. The GC-C-IRMS standard (Chiron International Standards, Laramie, WY) consisted of n-Undecane (C_{11}) with a $\delta^{13}C$ value of −26.11‰, n-Pentadecane (C_{15}) with a $\delta^{13}C$ value of −30.22‰, and n-Eicosane (C_{20}) with a $\delta^{13}C$ value of −33.06‰, each at 0.15 mg/mL in Cyclohexane. Injections of 1 μL of this standard were used to calibrate the reference CO_2 gas. The C_{11} peak in the isotope standard mixture was used to calibrate the reference cylinder to a user-defined value immediately prior to running all the samples in similar manner to Merritt et al. (30). The accuracy of the method was confirmed using an IAEA sample of caffeine, which gave the correct isotopic value to within the error of the measurements (see Bulk isotope ratio section above for details).

Results and Discussion

Bulk Isotopic Analysis

Five replicate subsamples were taken from random sections of each sample for bulk isotopic analysis. The results, with 95% confidence intervals, are as follows: A = −27.168 ± 0.03‰, −28.075 ± 0.02‰, −28.197 ± 0.09‰, −27.446 ± 0.04‰, and −32.433 ± 0.08‰. According to West et al. (23) our results indicate that marijuana sample E is consistent with indoor growth (as was known for this sample), whereas the four aged samples are more consistent with shade or outdoor growth conditions. West et al. (23) showed that indoor grown marijuana samples have a $\delta^{13}C$ values greater than −32‰, whereas marijuana frown outdoors has $\delta^{13}C$ values less than −29‰.

Table 1 shows the *p*-values calculated from two-tailed *t*-tests of each pairwise comparison of the sample means. All five samples are distinguishable from one another at the 95% confidence level. However, samples C and D are only significantly different at the 95.2% confidence level. In these cases, the total ion chromatograms (TICs) provided by the single quadrupole could presumably provide greater confidence in excluding the samples as having common origins, especially through the use of principal component analysis (e.g., 6,14,15). As a proof of principal example, however, we were interested to learn how effectively the compound-specific isotope ratios of certain components could discriminate between the same samples.

TABLE 1—p-*Values based on two-tailed* t-*tests for the comparison of* $\delta^{13}C$ *values (vs. VPDB) of bulk marijuana samples. Cells with a white background are significantly different at the 95% confidence level.* N = 5 *for each sample.*

	p-Values			
	B	C	D	E
A	2.1×10^{-10}	4.8×10^{-6}	2.2×10^{-5}	1.6×10^{-9}
B	–	4.8×10^{-2}*	8.4×10^{-7}	1.7×10^{-8}
C	–	–	5.2×10^{-6}	2.2×10^{-12}
D	–	–	–	4.9×10^{-11}

VPDB, Vienna Pee Dee Belemnite.
*Significance level = 95.2%.

Compound-Specific Isotopic Analysis

Our system allows for a single sample injection that can be analyzed by two separate detection systems simultaneously. For simplicity, the single-quadrupole mass spectrometer results are presented here as (TICs). The GC-IRMS results are presented as selected ion chromatograms and as real-time *m/z* ratios of 44/45. The four aged samples contained CBN as the major or second most abundant cannabinoid. CBD was the most abundant cannabinoid in samples C and D, but was only a trace component in samples A and B. Because of the age of samples A–D, THC was not detectable at significant levels. Conversely, sample E contained mainly THC and almost no CBN and CBD. The absence of comparable cannabinoids between sample E and the other four samples prevents any statistical comparisons; the samples can easily be excluded as common in origin based on their chemical composition, alone.

Example TICs from the single quadrupole MS and an example of a head-to-tail comparison for CBN is shown in Fig. 2. The head-to-tail comparison of CBD for samples C and D can also be seen in Fig. 2. The retention index and fragmentation pattern of all four samples clearly identify CBN as the major component with probability scores from the NIST library ranging from 70.9 to 84.5%. CBD is also abundant in samples C and D, with NIST scores ranging from 57.4 to 70.0%, respectively. The fragment ions that are most commonly used to identify CBN are *m/z* 238, 295, and 310. For CBD, the major fragments are *m/z* 174, 231, and 314 (5). The results from the single-quadrupole mass spectrometer provide a category A or confirmatory method of analysis according to SWGDRUG guidelines (31). The IRMS can then be used to further discriminate the unknown sample by assigning isotope ratios to the individual components within the sample. An even greater degree of discriminating power in establishing or excluding potential common sources of drugs would be possible by acquiring the N, H, or O isotope ratios (32), by chemometric analysis (33), or by elemental analysis of the metal impurities (34).

Isotopic Analysis of Cannabinol

The pooled average isotopic ratios for CBN in each sample, with errors reported as ±1 SD, are as follows: A = −29.33 ± 0.05‰, B = −30.01 ± 0.10‰, C = −30.31 ± 0.14‰, and D = −29.90 ± 0.17‰. The 95% confidence intervals are as follows: A = 0.03‰, B = 0.09‰, C = 0.11‰, and D = 0.09‰. To provide two examples, the simultaneous IRMS outputs for single sample injections of marijuana extracts A and D are displayed in Fig. 3. Our primary interest was to establish the sources of variation in $\delta^{13}C$ values for marijuana cannabinoid CBN for possible source matches. Three aliquots of each sample were analyzed four times each to establish the variation of $\delta^{13}C$ values in each marijuana sample (sampling variance). The replicate levels of each aliquot were subjected to a Grubbs test to determine whether any outliers existed. Based on the

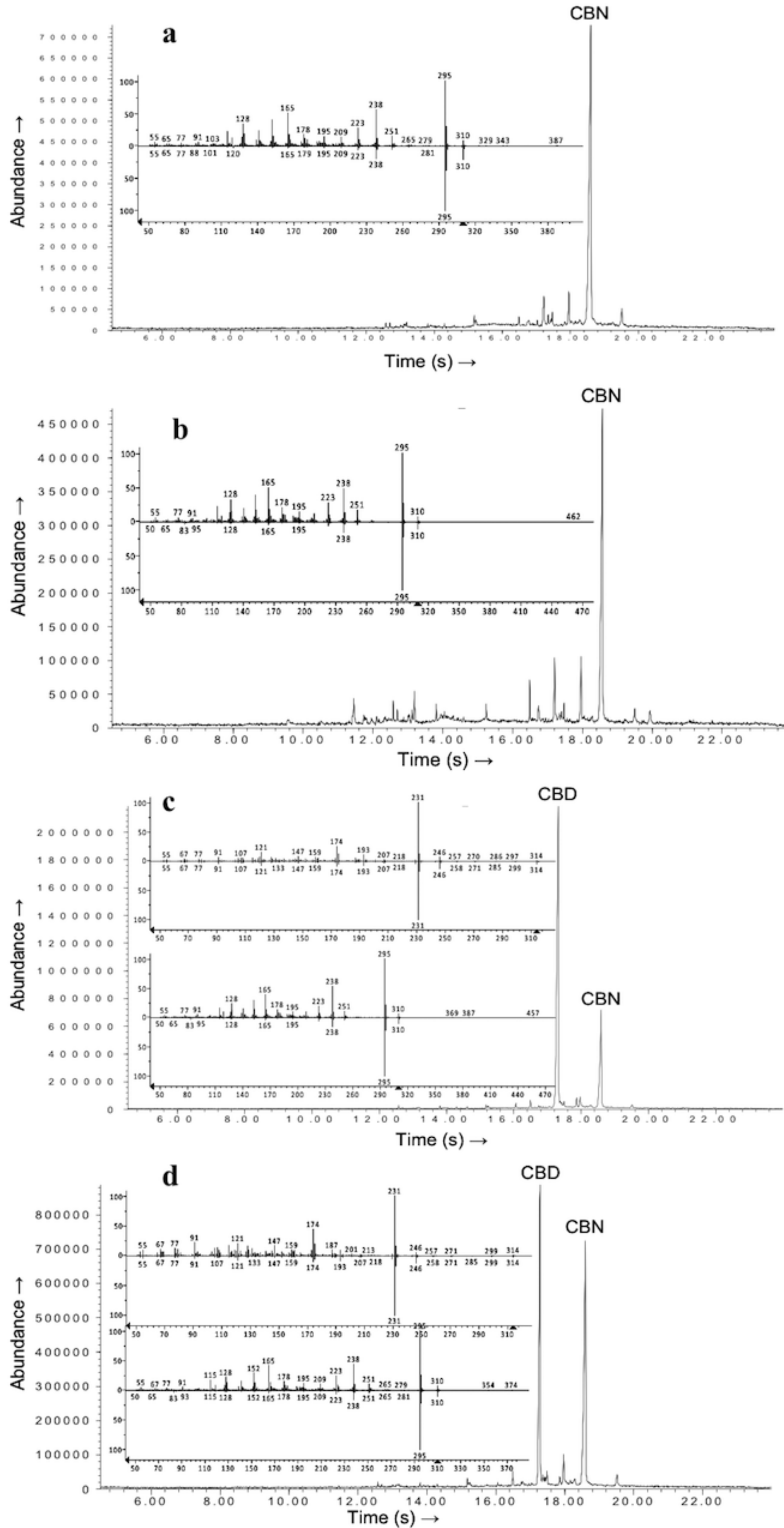

FIG. 2—*Examples of total ion chromatograms and mass spectra with the NIST head-to-tail library output of (a) sample A, (b) sample B, (c) sample C, and (d) sample D. Samples A and B contain mostly cannabinol (CBN) and samples C and D contain both cannabidiol and CBN.*

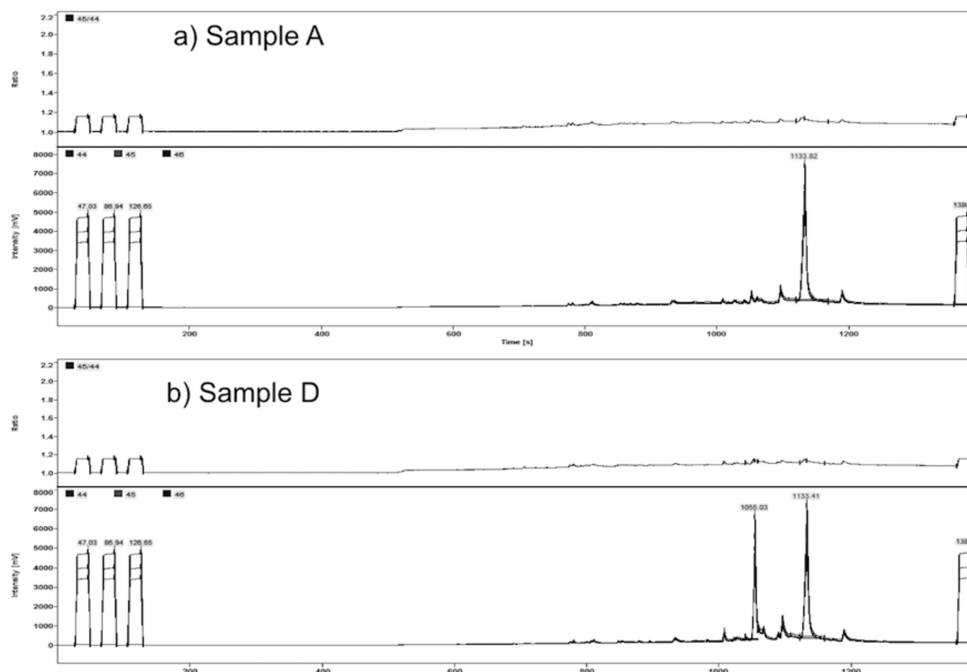

FIG. 3—*Examples of a concurrent isotope ratio mass spectrometry output acquisition for the same injections shown in Fig. 2: (a) sample A and (b) sample D.*

95% confidence level hypothesis test, six outliers were identified as potential outliers. These six outliers were the first six of the *c*. 48 chromatograms that were collected in the analytical sequence. The significantly different results of the first six injections of the day indicate a source of bias in the results, possibly from the freshly generated oxidation tube. A similar result was observed when repeating the entire sequence on a separate day. Fewer or no outliers have been noted in more recent studies when standards are run multiple times through the regenerated oxidation tube before initiating an analytical sequence.

The bar graph in Fig. 4 shows the isotope ratio results for CBN in each of the aliquots from the four samples. The separation between the different samples of marijuana can be visualized with error bars showing the 95% confidence intervals for each aliquot that range from 0.03 to 0.11‰. ANOVA was employed to separate the sampling variance from the measurement variance. The measurement variance is defined as the random error from all the instrumental sources. The sources of error include the injection, GC separation, oxidation, ionization, *m/z* separation, ion detection, signal amplification, integration, and data manipulation for the samples, in addition to the same random errors in the reference gas analysis. Sampling standard deviation refers to the variation between subsample means of each sample. Sampling standard deviations varied from 0.006 to 0.049‰ (average = 0.03‰) for the four aged samples. The measurement standard deviations varied from 0.11 to 0.22‰ (pooled average = 0.14‰ for CBN). Based on these values, the measurement error is approximately eight times larger than the between-subsamples (sampling) error. These results indicate that the marijuana samples were relatively homogeneous and that most of the error in the results is owing to instrumental sources of error in the IRMS. Our errors for replicate analyses of each sample are consistent with the literature and specifications for the instrument.

Based on the results of ANOVA, five of the six pairwise comparisons yield significant difference between each of the sample means at the 95% confidence interval. These results were further confirmed by performing *t*-test comparisons of the pooled sample

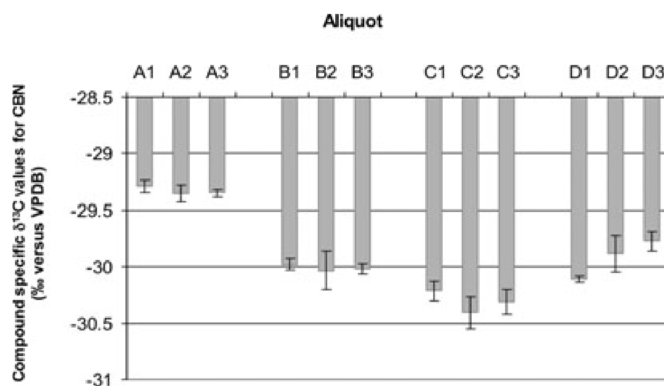

FIG. 4—*Average $\delta^{13}C$ values of cannabinol of four cannabis plant extracts: (a) sample A, (b) sample B, (c) sample C, and (d) sample D. Results represent average of three aliquots of each sample with quadruplicate sampling. Error bars indicate 95% confidence intervals. VPDB, Vienna Pee Dee Belemnite.*

means, the results of which are provided in Table 2. Sample A can be distinguished at the 95% confidence level from samples B, C, and D. Samples B and C and samples C and D can be similarly distinguished from one another at the 95% confidence interval. The sample means of samples B and D are not significantly different and therefore cannot be excluded as having a potential common source based on the $\delta^{13}C$ values of CBN alone. However, the relative abundance of CBD relative to CBN could presumably be used to further discriminate the two samples in this example (14,15). Visual inspection of the TICs obtained on the single-quadrupole mass spectrometer shows that the relative composition of other minor constituents are very different, which suggests that the two samples are in fact of different origin.

Figure 5 summarizes each of the isotope ratio measurements obtained for each sample. These values include bulk isotope ratios (all samples), CBN isotope ratios (samples A through D), CBD isotope ratios (samples C and D), and THC isotope ratios for sample

TABLE 2—p-*Values for the comparison of* δ^{13}C *values (vs. VPDB) of cannabinol extracted from marijuana samples based on two-tailed* t-*tests. Cells with a white background are significantly different at the 95% confidence level.* N = 12 *for each sample.*

	p-Values			
	B	C	D	E
A	1.8×10^{-13}	2.1×10^{-13}	3.6×10^{-8}	N/A
B	–	3.3×10^{-6}	$1.2 \times 10^{-1*}$	N/A
C	–	–	3.6×10^{-6}	N/A
D	–	–	–	N/A

VPDB, Vienna Pee Dee Belemnite.
*Significance level = 88%.

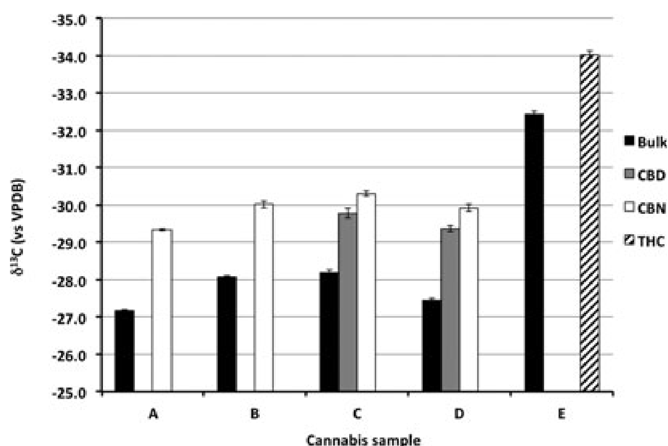

FIG. 5—*Comparison of bulk and compound-specific* $\delta^{13}C$ *values of five cannabis plant extracts: Results represent average of three aliquots of each sample with quadruplicate sampling (* N = 12*). Error bars indicate 95% confidence intervals. VPDB, Vienna Pee Dee Belemnite.*

E only. The cannabinoids all show more negative δ^{13}C values than the bulk measurements indicating that the cannabinoids are depleted in ^{13}C relative to the bulk plant matter. Paired differences between the bulk measurements and CBN measurements for the pooled values for each sample provided a consistent difference between CBN and the bulk values of 2.2‰, as seen in Fig. 5.

Isotopic Analysis of Cannabidiol

Samples C and D contained levels of CBD that were large enough for isotopic determination. The pooled average isotopic ratios for CBD in each sample, with errors reported as ±1 SD (N = 12), were −29.17 ± 0.14‰ and −29.97 ± 0.23‰, respectively. The 95% confidence intervals for the means were 0.08‰ and 0.13‰, respectively. A *t*-test between the mean δ^{13}C content of CBD in sample C and D showed a significant difference at the 99% confidence interval ($p = 5.8 \times 10^{-9}$), thereby confirming the results of the CBN comparisons that the sample means are significantly different. In this, and many other cases, the compound-specific isotope analysis provides a higher degree of confidence than the bulk isotope analysis for the same samples.

We also compared the δ^{13}C values for CBN and CBD within samples C and D. Samples C and D each showed significant differences in the isotope ratios of these two degradation products. For sample C, the CBN and CBD δ^{13}C values differed by 0.75‰ ($p = 1 \times 10^{-10}$, two-tailed *t*-test) and for sample D, the values differed by 0.34‰ ($p = 3 \times 10^{-4}$, two-tailed *t*-test). The significant difference in isotope ratios between CBN and CBD in each of the samples is surprising given that both degradation products contain the same number of carbon atoms as the THC from which they presumably originated. Although our sample size is very small, these differences suggest that CBN and CBD are either formed or degraded (or both) in a manner that causes fractionation differences between them.

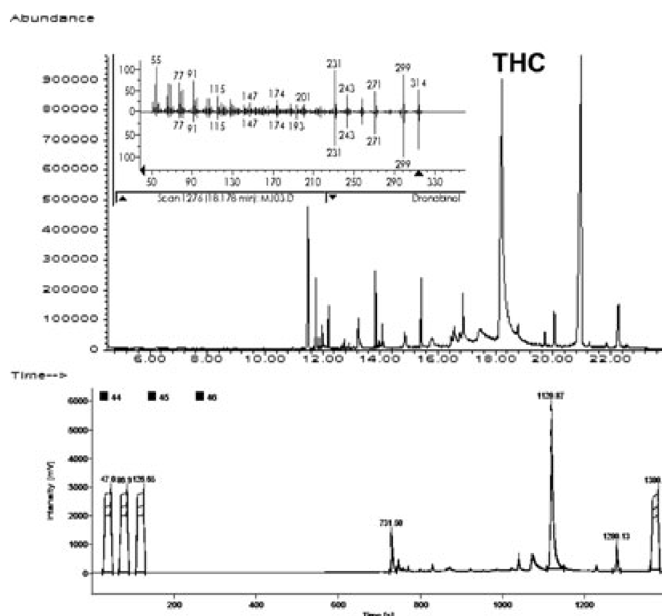

FIG. 6—*Example of concurrent data for an extract from fresh cannabis: (a) total ion chromatogram and mass spectrum of the* Δ^9*-tetrahydrocannabinol (THC) peak showing a head-to-tail comparison with a NIST spectrum of THC standard (Dronabinol) and (b) concurrent isotope ratio mass spectrometry output acquisition for the same injection.*

Isotopic Analysis of Tetrahydrocannabinol

Examples of a single quadrupole result and IRMS result from the same injection of sample E are shown in Fig. 6. Three subsamples of this plant matter were each measured in quadruplicate (N = 12) to provide compound-specific δ^{13}C ratios for THC of −34.03 ± 0.03‰ (95% confidence interval). We also measured the bulk δ^{13}C isotope ratio for this sample to be −32.45 ± 0.03‰ (95% confidence interval, N = 5). These results indicate that, at least for this sample, the carbon isotope ratios for the extracted THC are significantly different in ^{13}C content than the bulk leaf matter from which the THC was extracted ($p = 2 \times 10^{-13}$, two-tailed *t*-test). The difference between the average THC and average bulk value is 1.62‰, which is very close to the average difference between CBN and bulk values (1.71‰) for the other four samples.

Comparison of Bulk and Compound-Specific Analyses

Bulk isotope analysis has the obvious advantage that it can be performed on almost any substance regardless of the substances chemical properties. Bulk isotope analysis typically requires less sample preparation and is somewhat faster than the compound-specific analyses on the GC-IRMS system. In these regards, it is certainly more favorable than compound-specific IRMS. In contrast, compound-specific IRMS measurements has the benefit that it can be performed on much smaller sample sizes and can therefore be applied to trace levels and swabbed samples.

Regarding the performance of the two approaches, the pooled precision (1 SD) for bulk isotope measurements for all our

marijuana measurements was 0.06‰. The pooled precision (1 SD) for the compound-specific isotope ratio measurements was 0.13‰. These precisions are significantly different at the 95% confidence interval ($p < 0.05$, F-test). This result indicates that the EA-IRMS method has significantly better precision than the GC-IRMS method. Most of the error in the GC-IRMS method is known to stem from the measurement process and not from the extraction or sample preparation method (see CBN section for details).

For samples B and C, bulk IRMS measurements only distinguished between the two samples at a confidence level of 95.2% (Table 1). However, compound-specific IRMS of CBN from extracts of the same samples provided discrimination between the two samples at the >99.999% confidence level (Table 2). In contrast, samples B and D were found to be very significantly different using bulk IRMS measurements but not significantly different at the 95% confidence interval using the compound-specific values for CBN. Sample B did not contain significant quantities of CBD so could not be compared with the CBD measurements for sample D. One would expect the compound-specific isotope method to provide better discriminating power when multiple independent values are available for comparison between two different samples.

The National Academy of Science has recommended in a report to Congress that the forensic science community in the United States use statistical validation and confidence reporting when treating the comparison or measurement of forensic samples (35). Analyses involving the measurement of $\delta^{13}C$ values of specific compounds in a mixture, and the comparisons thereof, clearly fall within the guidelines of statistical and confidence reporting. However, a significantly larger database of frequency of $\delta^{13}C$ values of natural and synthetic drugs needs to be generated to determine the probability that two random samples have indistinguishable sample means. Without such a database, the evidentiary value of "matches" will not be as valuable or helpful for law enforcement as the value of exclusionary results.

The results presented here indicated that three of the abundant cannabinoids in aged or fresh cannabis samples are somewhat depleted in ^{13}C relative to the bulk plant matter. The individual cannabinoids vary from 1.6 to 2.4‰ more negative than the corresponding bulk isotope measurements. For the two samples in which more than one cannabinoid was measured, CBN and CBD showed $\delta^{13}C$ values that were significantly different from each other and from the bulk values ($p < 0.05$, two-tailed t-test). These results indicate that isotope fractionation is likely to occur during the degradation pathway of THC to CBN and CBD and/or in the subsequent degradation of CBN and CBD. In addition to confirming the presence of cannabinoids in plant matter, GC-MS/IRMS could also be applied to compound-specific cannabinoids from swabs of drug paraphernalia or from extracts of biological samples like urine and hair. With appropriate validation studies, such measurements could conceivably link a bulk source of a drug to a small quantity used or handled by an individual.

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