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CRIMINALISTICS*Helen W. Kreuzer*,^{1,†} *Ph.D.*; *Jason B. West*,^{1,‡} *Ph.D.*; and *James R. Ehleringer*,¹ *Ph.D.***Forensic Applications of Light-Element Stable Isotope Ratios of *Ricinus communis* Seeds and Ricin Preparations***

ABSTRACT: Seeds of the castor plant *Ricinus communis* are of forensic interest because they are the source of the poison ricin. We tested whether stable isotope ratios of castor seeds and ricin preparations can be used as a forensic signature. We collected over 300 castor seed samples worldwide and measured the C, N, O, and H isotope ratios of the whole seeds and oil. We prepared ricin by three different procedures, acetone extraction, salt precipitation, and affinity chromatography, and compared their isotope ratios to those of the source seeds. The N isotope ratios of the ricin samples and source seeds were virtually identical. Therefore, N isotope ratios can be used to correlate ricin prepared by any of these methods to source seeds. Further, stable isotope ratios distinguished >99% of crude and purified ricin protein samples in pairwise comparison tests. Stable isotope ratios therefore constitute a valuable forensic signature for ricin preparations.

KEYWORDS: forensic science, ricin, castor, *Ricinus communis*, stable isotope, sample matching, forensic signature

The seeds of the flowering plant *Ricinus communis*, commonly known as the castor plant, are the source of castor oil, a valuable commercial commodity, as well as the toxic protein ricin. Ricin is classified as a Schedule 1 controlled substance under the Chemical Weapons Convention, a Category B (biological agents/toxin) substance under the Biological Toxins Weapons Convention, and is also included as a Category B select agent set forth by the U.S. Department of Health and Human Services (42 CFR Part 73). The castor plant thus presents a conundrum: an important commercial agricultural plant that could potentially be used to produce a biological threat agent.

Castor is thought to have originated in Eastern Africa or India and is one of the oldest known commercial crops (1). Castor seeds, commonly known as castor beans, have been discovered in 4000-year-old Egyptian tombs and were used for oil to fuel lamps. In India, the oil was also used for lamps and medicinally as a laxative. Historically, the Chinese used castor oil for medi-

cine or in cooking. In the southern part of Russia, large castor plants were planted near buildings to provide shade and act as wind breaks (2). Castor is currently cultivated as an oilseed crop in tropical and subtropical regions throughout the world, and the plant has naturalized in warm regions around the world. Castor plants, which have a striking appearance, are also common as ornamental plants.

Ricin was investigated as an offensive weapon by the U.S. military after World War I, and other governments have reportedly explored its use (3). The toxin exerts its lethal effect by depurinating an adenine residue within eukaryotic ribosomal RNA, ablating a binding site for an elongation factor, and thereby inhibiting protein synthesis (4). The lethal dose has been estimated to be 5–10 µg/kg body weight, if the toxin is inhaled or injected (5). The median lethal oral dose in mice is *c.* 1000 times greater than the inhaled or injected lethal dose, and the lethal oral dose for humans has been estimated to be 1–20 mg ricin/kg body weight (6). Despite this potential lethality, actual cases of poisoning from ingesting castor seeds have resulted in far fewer deaths than might be anticipated (7).

Much of the concern over ricin as a potential biological threat agent stems from the widespread availability of castor seeds. Numerous procedures for purification and partial purification of ricin from castor seeds have been published. Approximately 50% of the weight of a castor seed is oil. Ricin comprises about 1.5% of the oil-free meal and, as a water-soluble protein, is not extracted when oil is removed by organic solvent extraction (2). A process for producing large volumes of ultrapure material patented by the U.S. Army (8), as well as research laboratory procedures based on chromatographic separation, can be found in the scientific literature (9). In addition to these highly technical procedures for preparing purified ricin, however, numerous “kitchen” methods for preparing ricin can be found in books

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characterized as “anarchist literature” and/or posted on the Internet (10–13). The most elementary of these procedures call for simply peeling and grinding castor beans into a paste. The next simplest procedure calls for peeling and grinding the beans, then removing oil from the resulting mash, usually by acetone extraction. These procedures are self-identified as “ricin” preparation methods and give rise to ricin-containing preparations of various levels of purity. They are easy to carry out and do not require sophisticated equipment.

In recent years, ricin-containing samples have been seized primarily in connection with biocriminal activity (14). Analytical methods that enable investigators to determine how the samples were prepared, to match seized samples to potential source materials, and to identify samples that may have been prepared by the same method using the same source materials would be welcome new additions to the tools available to investigators. Methods have been published for characterizing the procedure that might have been used to prepare ricin from castor seeds, such as by detecting residual solvent in the preparation or determining carbohydrate and fatty acid profiles in the sample (15,16). These methods achieve their stated purpose, but likely are not capable of differentiating samples prepared by the same method. Because of the wide potential growth range of castor seeds, we hypothesized that there would be considerable variation in the isotopic ratios of these seeds. As these isotope ratios would be inherent in the seed itself and independent of preparation method, they constitute an orthogonal forensic signature. Here, we report the isotope ratios of over 300 samples of castor seeds collected from worldwide locations spanning the climate zones in which castor plants can potentially grow. We measured the C, N, O, and H isotope ratios of whole seeds, castor oil extracted from the seeds, and three different types of ricin preparations, de-fatted seed pulp from which oil had been removed, a crude protein precipitate, and affinity-purified ricin, and evaluated the potential forensic utility of these isotope ratios.

Materials and Methods

The Castor Seed Collection and Sample Preparation

Castor seeds were collected from multiple locations in North America, South America, Europe, Africa, and Asia (Table 1). We used several different methods to assemble the collection: (i) we traveled to different regions and collected seeds directly from growers or distributors; (ii) solicited donations from people who either grew the seeds themselves or collected them from known locations; and (iii) sent seeds to cooperative colleagues who

TABLE 1—Growth locations of castor seeds collected and analyzed.

Country of Origin	Number of Samples
Brazil	29
Canada	3
China	21
Costa Rica	5
Ethiopia	2
France	4
Germany	2
India	28
Indonesia	1
Kenya	2
Suriname	6
Tanzania	6
United States	198
Total samples	307

planted them and sent us progeny seeds. Each sample of castor seeds in the collection was associated with the latitude and longitude of its growth location. The growth locations of the seeds in the collection are shown in Fig. 1, in which the regions of the world with average annual temperature $>7^{\circ}\text{C}$ are shaded. This average annual temperature corresponded to a region in North America that roughly overlaps the U.S. Department of Agriculture map of “hardiness zones” where castor is expected to grow (<http://usna.usda.gov/Hardzone/ushzmap.html>).

Safety and Regulatory Issues

Several components of castor beans were extracted in our laboratory at the University of Utah, including castor oil, whole bean paste, de-fatted bean mash, and crude protein. Castor seeds themselves are an unregulated agricultural commodity that can be freely purchased in the United States. However, the U.S. Centers for Disease Control and Prevention regulates laboratories working with purified ricin in quantities exceeding 100 mg. To stay below the regulatory level of the CDC, we never permitted more than 100 mg of active ricin to be in processing at any one time. A generous estimate of ricin content in a castor bean seed is 0.5% of the seed’s weight. Therefore, the total weight of all seeds in processing at any given time was limited to 20 g or less ($20\text{ g} \times 0.005 = 0.1\text{ g}$). Once samples were prepared, they were immediately inactivated.

Several methods for the inactivation of ricin have been published (17,18), and we used different methods for different types of materials. Ground whole beans and solvent-extracted bean mash were placed in capped glass vials and autoclaved at 121°C for 60 min. Extracted crude protein samples were inactivated by drying the aqueous samples in an oven at 90°C for 12–24 h. All work surfaces in direct contact with the active whole bean, bean mash, or crude protein were rinsed with $\geq 0.06\text{ N NaOH}$. Contaminated laboratory ware was soaked in either 10% bleach or $\geq 0.06\text{ N NaOH}$ for at least 30 min. Solid waste materials were autoclaved at 121°C for 60 min.

Ground Whole Seeds

To produce whole seed paste, we homogenized five seeds per sample using a Retsch MM200 machine (Retsch GmbH & Co., Hann, Germany). Samples were homogenized in multiple rounds, adding 1–2 beans every 30 sec until a homogenous paste was achieved.

Castor Oil

Castor seeds were soaked for 1 h in 3 N NaOH to soften the husks (the solution did not penetrate the husks), rinsed in water, and then peeled. The peeled seeds were homogenized in a Retsch MM200 machine and then exposed to acetone, hexane, or a mixture of the two, for 48 h. Subsequently, the mixture was centrifuged, the supernatant collected, and the solvent evaporated, leaving the oil behind.

De-Fatted Castor Seed Pulp

De-fatted castor seed pulp consisted of the seed solids remaining after solvent extraction of castor oil as described above. Castor seed pulp should be void of all oil and consist of primarily the carbohydrate and protein components of castor seeds. We prepared and determined the C, N, O, and H isotope ratios of de-fatted pulp from 256 samples of seeds.



FIG. 1—Growth locations of castor seeds collected and analyzed. Dark shading highlights regions with average annual temperature $\geq 7^{\circ}\text{C}$, where castor plants could reasonably be expected to grow. MAT, mean annual temperature.

Crude Protein Precipitate

Crude protein was extracted using a method provided by the Federal Bureau of Investigation (19), which generally corresponded to a “ricin preparation method” as described in Harber (11). Briefly, the husks of castor seeds were removed as described above, and most of the oil was removed by pressing the seeds overnight between layers of paper towels topped with weights. After oil removal, the castor bean seeds were soaked in a 10% wt/vol NaCl solution for 48 h. Epsom salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) was then added to the NaCl solution to precipitate the proteins. After 1.5 h, the precipitate was collected by centrifugation, rinsed three times with distilled water, and heated at 90°C for 12–24 h to inactivate the protein and dry the pellet. We prepared and determined the C, N, O, and H isotope ratios of crude protein precipitate prepared from 255 samples of seeds.

Affinity-Purified Ricin

Highly purified ricin was prepared at Oak Ridge National Laboratory from 75 samples of seeds under a subcontract. The procedure was as follows: Seeds were soaked in 3 N NaOH for 1 h to overnight. The base was neutralized, the seeds rinsed with copious amounts of water, and the seed coats were manually removed. Shelled seeds were ground with phosphate-buffered saline solution using 4 mL buffer per 1 g seeds, and the suspension was centrifuged at 4°C for 20 min at $10,000 \times g$. The suspension separates into three layers, debris at the bottom, aqueous, ricin-containing layer in the middle, and oil at the top. The middle layer was recovered, re-centrifuging if needed to complete the oil removal. Ice-cold acetone was added to the middle layer until a precipitate formed (~90% acetone). The precipitate was recovered by centrifugation at 4°C for 20 min at $10,000 \times g$ and resuspended in 10 mM Tris-HCl buffer (pH 7.7), using 2 mL buffer for each gram of seeds. The ricin in this solution was purified by affinity chromatography. Briefly, 1 mL of modified Sepharose 4B beads per 5–6 mL solution was added and the solution gently rotated for 1 h. Ricin adsorbs to the beads. The beads were recovered by centrifugation at $500 \times g$, washed with Tris buffer, and then put into a syringe plugged with glass wool and fitted with a stopcock to form a column. The column was washed $2 \times$ with Tris buffer, then the ricin was eluted with two column volumes of 0.12 M D-galactose in 10 mM Tris-HCl (pH 7.7). This solution was de-salted on a Sephadex G25 column. The column was washed with $2 \times$ bed

volumes water. For a 3-mL column, the first 0.9 mL eluate was discarded and the next 1.8 mL was collected. Protein concentration was monitored by absorbance at 280 nm on a spectrophotometer. The final solution of near-pure ricin was dried down and denatured by heating at 80°C for 10 min. These dried samples were subjected to isotope ratio analysis.

Isotope Ratio Measurement

All solid castor bean component and oil samples were weighed on a Sartorius MC 5 microbalance (Sartorius AG, Goettingen, Germany) and analyzed for C, N, O, and H stable isotope content. The exceptions were oil samples, which do not contain nitrogen and were analyzed for C, O, and H isotopic content only. Stable isotope content is measured as a ratio, R (e.g., $^{13}\text{C}/^{12}\text{C}$), and reported as a delta (δ) value where $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000\text{‰}$. In this equation, R_{sample} is the measured isotope ratio of the sample and R_{standard} is the isotope ratio of an internationally recognized standard. The standard for C isotope ratio measurement is Vienna PeeDee Belemnite, for N is air, and for O and H is Vienna Standard Mean Ocean Water (20). Samples were normalized to these scales using single-point offset corrections to internal laboratory reference materials (cellulose for O and H and yeast for C and N) that had been previously calibrated against internationally distributed standards.

Solid samples (whole seeds, seed mash, and crude protein) to be analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes were weighed into tin capsules (3.5×5 mm). Two mg of each sample were weighed along with in-house reference materials for each analysis. Oil samples (2 mg) analyzed for $\delta^{13}\text{C}$ were weighed into thicker tin capsules (3.5×9 mm) along with the yeast reference and an additional, commercially available castor oil. Samples were stored at room temperature and analyzed on a Finnigan MAT Delta S Isotope Ratio Mass Spectrometer (IRMS, Bremen, Germany) controlled by Isodat NT software. The mass spectrometer was coupled to a Carlo Erba Elemental Analyzer 1108 (EA) with a 3 m Poraplot Q column through a ConFlo II Interface. The instrument precision of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements is 0.2‰, as shown by analyses of our in-house yeast reference material over several years.

For $\delta^{18}\text{O}$ and $\delta^2\text{H}$ analysis, 0.150 mg solid samples were weighed into silver capsules (3.5×5 mm). Aliquots of our in-house cellulose reference material were included in each sample set. To analyze castor oil for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, we weighed

0.185 mg oil into silver capsules (2×5 mm). Our in-house cellulose reference material and the commercially available castor oil were included in each of these sample sets also. Prior to use, the silver capsules were baked for 15 min at 500°C in a muffle furnace to remove any oxidation products. Unused, baked capsules, and weighed samples were stored under vacuum. All samples were run on a Finnigan Delta plus XL IRMS controlled by Isodat NT software. The IRMS was equipped with a temperature conversion/elemental analyzer (TC/EA) with a 1-m 5\AA molecular sieve column. The instrument precision of our $\delta^{18}\text{O}$ measurements is 0.2‰ , and of $\delta^2\text{H}$ measurements is 2‰ , as determined by analysis of our reference material over several years.

Results

Figures 2–5 show the frequency distributions of C, O, H, and N stable isotope ratios for ground whole seeds and seed components ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$, and $\delta^{15}\text{N}$, respectively). Figures 2–4 report the isotope ratios of whole seeds, seed pulp after the oil was extracted, the castor oil itself, and that of a crude protein extract (see Materials and Methods for preparation details). Figure 5 presents N isotope ratios for whole seeds, de-fatted pulp, crude protein, and highly purified ricin.

An important potential forensic use of ricin isotope ratio data would be to determine whether a given batch or source of castor seeds could have been used to produce a particular ricin sample, such as if a suspect in a ricin-related case had castor plants growing in his or her yard. Our samples of de-fatted seed pulp, crude protein precipitate, and affinity-purified ricin represent ricin preparations following various methods in the anarchist and laboratory literature. Regressions of the N isotope ratios of de-fatted seed pulp and crude protein precipitates, as well as those of affinity-purified ricin, against the N isotope ratios of the source seeds showed strong correlations (Fig. 6). Significant

positive correlations for C, H, and O were also observed, with somewhat higher levels of unexplained variation (data not shown). Our results suggest that the N isotope ratios of these preparations most closely track those of whole seeds. Other sources of variation in the C, H, and O results likely include biochemical and physiological effects that will be explored in future efforts. While a correspondence between the N isotope ratios of a ricin sample and suspect castor seeds could not alone prove that the seeds were the source, it would support the association. Conversely, a lack of correspondence would provide strong evidence for ruling out the seeds as the source material.

Another potential forensic use of ricin stable isotope ratio data would be to determine whether two samples, perhaps obtained in different locations, could have been produced from the same source beans. In this case, the stable isotope ratios of the two samples should be indistinguishable. We evaluated the utility of stable isotope ratios to distinguish ricin samples prepared by the same method using our data sets for defatted bean pulp, crude protein precipitates, and affinity-purified protein. Within each group prepared by the same method, every sample was prepared from different source beans. We compared the C, N, O, and H isotope ratios of every sample within a preparation type against those of every other sample and measured the frequency with which random pairs from different sources were indistinguishable based on their isotope ratios. We defined isotope ratios as being indistinguishable when the two values being compared were within two standard deviations of one another, using the long-term instrument precision intervals cited above as one standard deviation. This requirement provided a confidence interval of *c.* 95% (21). For C, N, and O isotope ratio measurements, the instrument precision is 0.2‰ , and therefore, two values had to be within 0.4‰ of each other for them to be declared indistinguishable. For two H isotope ratios to be declared indistinguishable, they had to be within 4‰ of each other using the same

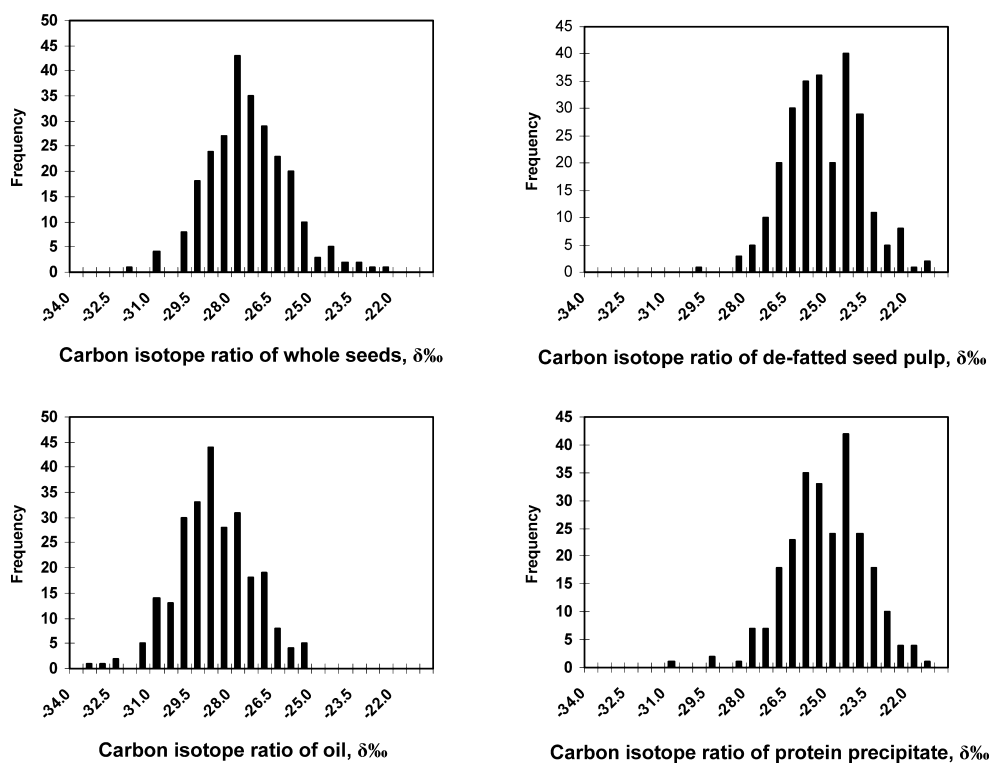


FIG. 2—Distribution of C isotope ratios of whole seeds and seed components.

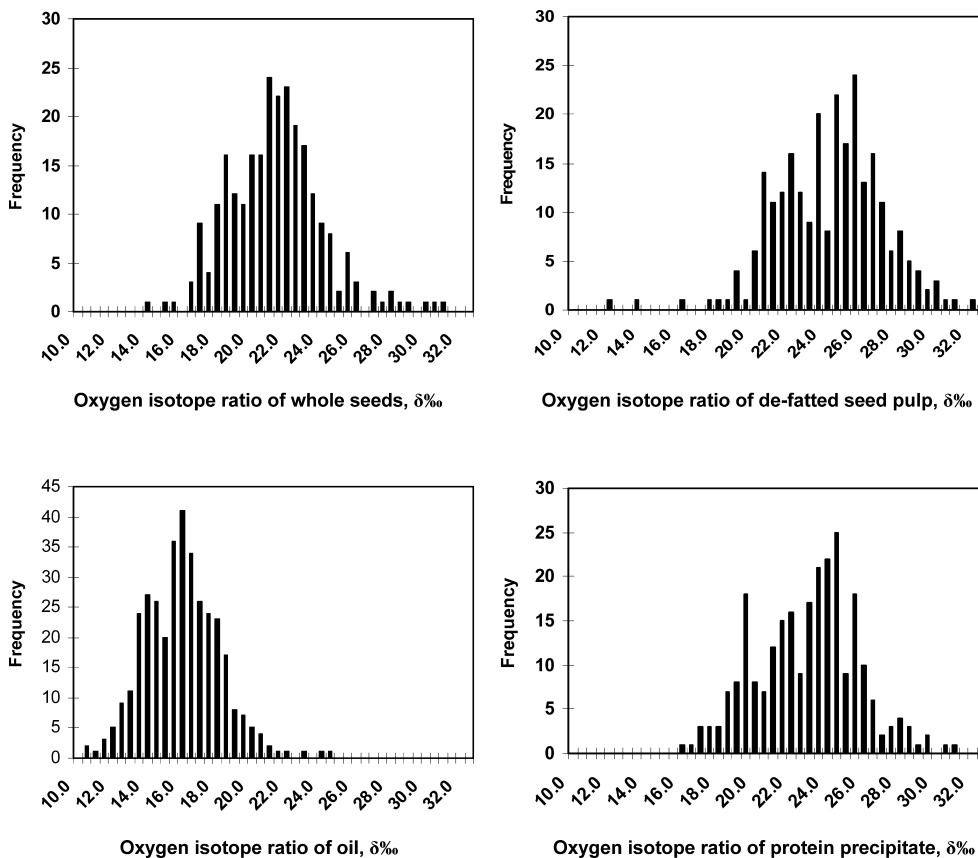


FIG. 3—Distribution of O isotope ratios measured in whole seeds and various seed components.

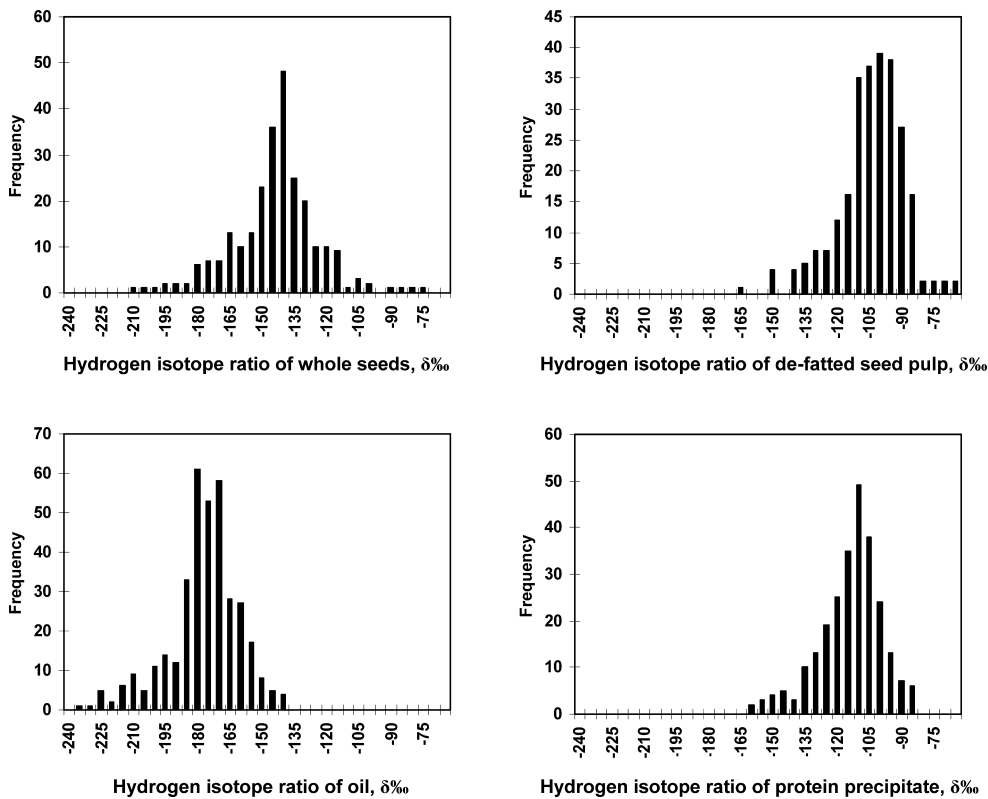


FIG. 4—Distribution of H isotope ratios measured for whole seeds and seed components.

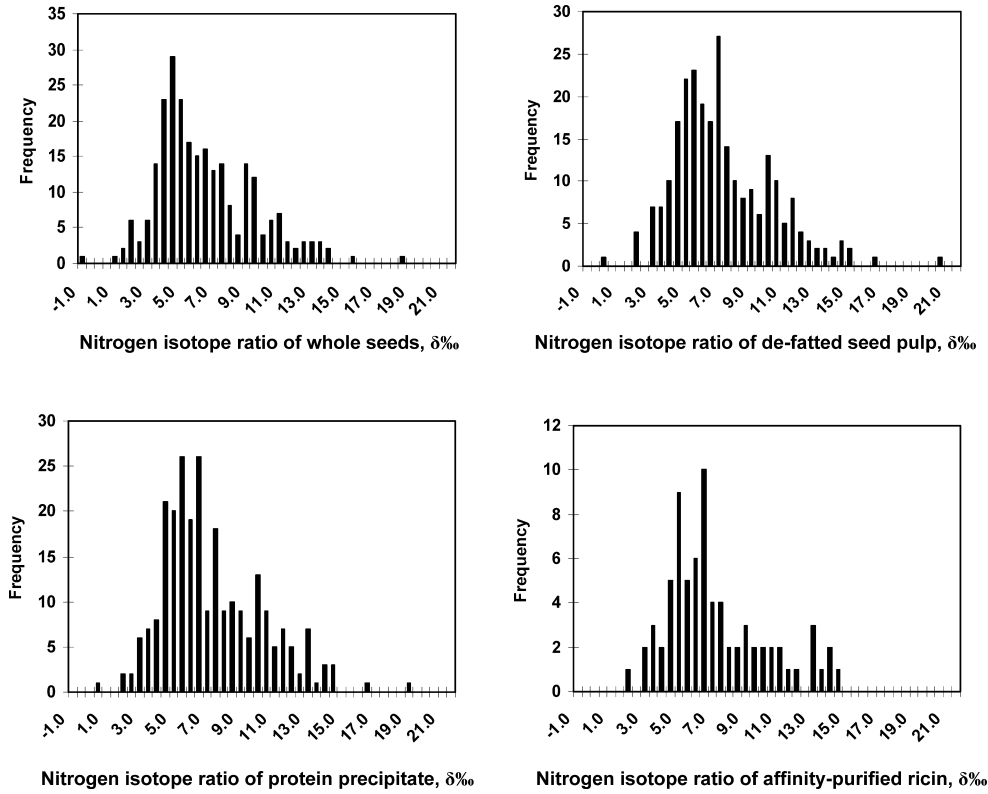


FIG. 5—Distribution of N isotope ratios measured for whole seeds and various seed components. Oil is not shown in this figure because it does not contain N.

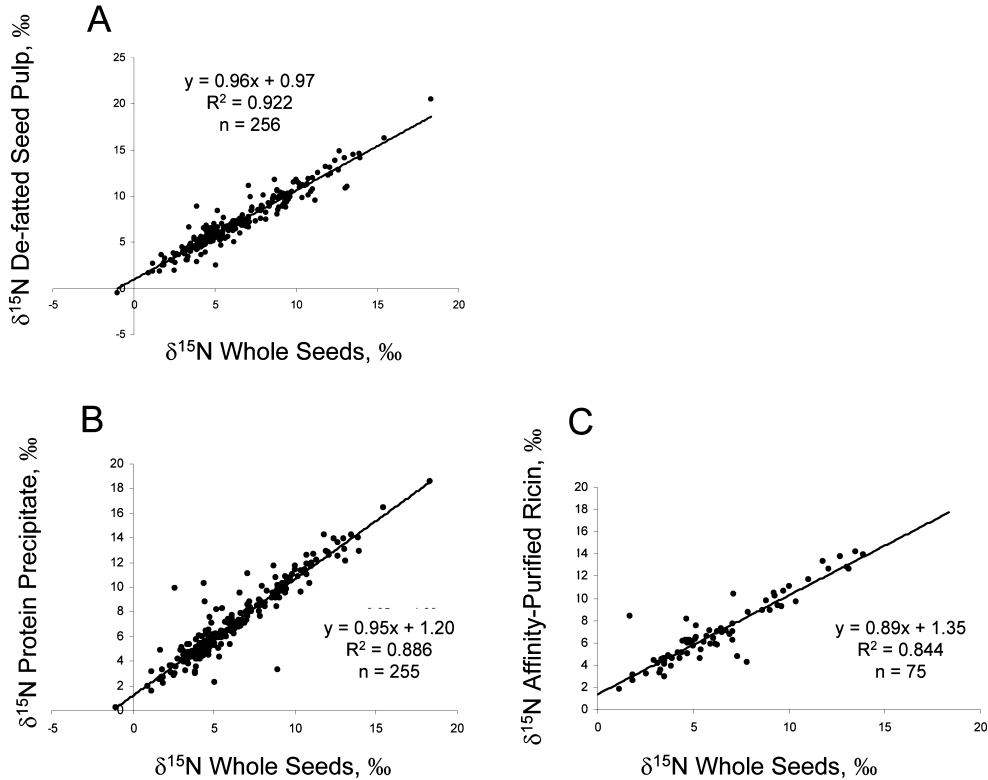


FIG. 6—Regression of N isotope ratios of ricin preparations on those of source castor seeds. Panel A, de-fatted seed pulp preparations. Panel B, crude protein precipitates. Panel C, affinity-purified ricin.

criteria. For two samples to be declared indistinguishable, all four isotope ratios had to meet the definition of indistinguishable. These criteria derive from random variation inherent to

current processing and instrumentation methodology and do not include other sources of variation that might be observed or that might be of interest for a specific seed source (e.g., genetic or

physiological variation or mixtures of populations). The amount of variation these additional factors might introduce is likely to be population specific and the degree of its importance dependent on the individual samples or populations under consideration. The ranges reported here should therefore be interpreted as conservative estimates of similarity.

When we tested the stable isotope signatures of the de-fatted bean pulp samples (256 total samples), only 201 pairs of samples of 32,640 possible pairs $[(n^2-n)/2]$, or 0.6%, were indistinguishable. Of the 255 crude protein extracts, only 208 pairs of samples of 32,385 possible pairs, or 0.6%, were indistinguishable. Of the 75 samples of affinity-purified ricin, only five pairs of samples of 2775 possible pairs, or 0.2%, were indistinguishable in all four isotope ratios; thus, >99% of the samples could be distinguished from one another based on a comparison of C, N, O, and H isotope ratios.

Discussion

Isotopic Content of Seeds and Seed Fractions

The whole seeds showed ranges of over 9‰ in C isotope ratios, over 19‰ in N isotope ratios, over 130‰ in H isotope ratios, and over 16‰ in O isotope ratios. These ranges reflect the variety of environments in which the plants were grown with some unknown but likely small contributions from differences in physiology or morphology among individuals (22,23). Given that our collection contained seeds from growth regions representing the range of climate zones in which *R. communis* could be expected to grow, this set of data may represent most of the isotopic variation that would be commonly observed in *R. communis*.

C, O, and H isotope ratios in plants are influenced by climate and plant response to climatic variation. *R. communis* fixes CO₂ via the C₃ pathway, and the C isotope ratios we observed for our samples are consistent with previously measured values for C₃ plants (24). The C isotope ratio of a given plant species is modulated by climatic factors such as relative humidity and water stress (25–27), and the range of values we observe likely reflect differences in these factors across growth locations. Plant tissue O and H atoms are derived from environmental water that is then modified during plant growth. Variation in the isotopic content of plant water can result from differences in the isotopic content of local precipitation, leaf water isotopic enrichment during transpiration, and the extent to which leaf stomata remain open to permit diffusion of water into the leaf. These latter two factors are dependent on climate, which can also promote evaporative enrichment of surface water. The synthesis of organic compounds for growth and reproduction is also associated with isotopic changes, and thus, the δ²H and δ¹⁸O of all plant tissues and materials derived from plant tissues reflect the influence of precipitation inputs, soil moisture changes, climatic influences on plant physiology, and biochemistry associated with plant growth (28–30). These effects are likely to be similar for plants growing in similar geographic locales, and understanding these influences on *Ricinus* δ²H and δ¹⁸O is an area of active research that is beyond the scope of the current study. The nitrogen isotopic content in plants is a function primarily of the N sources available, especially among individuals of the same species, as in this study (31). The wide range in N isotopic content thus likely reflects differences in application and type of fertilizer (if any).

The relationships we observed between the isotopic content of whole seeds, oil, de-fatted seed pulp, and the two protein prepa-

rations were not surprising. Castor seeds are composed of c. 50% oil, with the remainder being mostly carbohydrate and protein (2,32). The biosynthetic process by which fatty acids are synthesized has been shown to discriminate against ¹³C, resulting in the isotopic depletion of lipids versus other types of tissue (33–35). This relationship is apparent in Fig. 2. The samples of castor oil displayed, in aggregate, lower ¹³C content than whole seeds. Mass balance then predicts that the seed residue left over after oil extraction would be enriched in ¹³C with respect to both the whole seeds and the oil, and this is indeed what was observed. The residue is mostly protein and carbohydrate; it was therefore not surprising that the C isotope ratios of crude protein preparations were also higher than those of the oil or whole seeds.

The O and H isotope ratio distributions among different seed components resembled those of C isotope ratios (Figs 3 and 4). The oil is depleted in ¹⁸O and ²H, as has been reported by others who have measured the isotopic content of plant lipids and total plant biomass (e.g., [36]). Again, as predicted by mass balance, the de-fatted seed pulp and protein fractions were enriched in these isotopes compared with the whole seeds.

As castor oil contains no N, the N isotope ratio of de-fatted seed pulp left behind after oil removal is very similar to that of the whole seeds. Most of the N would presumably be present in protein (as opposed to amino sugars), and therefore, one might predict that the distribution of N isotope ratios in whole seeds and de-fatted pulp would be very similar (Fig. 5). Perhaps not surprisingly, the N isotope ratios of affinity-purified ricin also tracked that of the whole seeds, seed pulp, and crude protein, although significant variation in δ¹⁵N of different amino acids has been observed (37,38).

Stable Isotope Ratios as Potential Forensic Signatures for Ricin

Applications of stable isotope ratios in forensic science include sample matching and differentiation, correlation of products to source materials, and association of plant or animal material with geographic regions of origin (39–45). The data presented in this study demonstrates that stable isotope ratios of ricin preparations and castor seeds can be used for the first two of these applications. The N isotope ratios of ricin prepared by three different methods (yielding ricin protein of increasing purity) correlated strongly to those of the source beans. Thus, comparing the N isotope ratios of a forensic sample to those of suspected source seeds could help rule out those seeds as potential sources.

Our data also demonstrate that the C, N, O, and H isotope ratios of a castor preparation constitute a powerful signature for sample matching. The combination of these stable isotope ratios discriminated more than 99% of samples of de-fatted seed pulp, of crude protein extracts, and of affinity-purified ricin. Because every sample in a category of ricin samples was prepared by the same method, the preparation signatures of the samples in one category, such as acetone headspace concentration or lipid and carbohydrate profiles (15,16), should all be quite similar. Isotope ratios of ricin samples could therefore serve as an orthogonal forensic signature for sample discrimination.

Finally, stable isotope ratios of plant material have been used to determine the geographic origin of the material (44–46). The most direct link of the castor seed data to geographic regions would be expected to arise from linking O or H isotope ratios to those of local plant source water, which exhibits predictable spatial variation, although C and N isotope ratios have also been

used for geographic origin assessments (44). West et al. (47) have used a model of the H isotope ratio of castor oil derived from controlled growth experiments that relates it to source water, and projected the results of this model onto a geospatial grid, using predicted local water isotope ratios generated by Bowen et al. (48,49). The predicted values for castor oil that corresponded to sample locations in our collection were then compared with the measured values of the oil from those seeds. The predicted values and the measured values correlated closely, with all points falling close to the 1:1 line, indicating that the H isotopic content of castor oil could be useful in geographic sourcing. However, as many locations throughout the globe have similar precipitation isotope ratios, predictions based on H isotope ratios alone could only be used to eliminate general areas from consideration. The isotope ratios of elements such as Sr and Pb are related to geography and thus have been used to assess region of origin of migratory species as well as archaeological materials (50–54). It is possible that combining data such as Sr isotope ratios with H isotope ratios would further and significantly restrict potential regions of origin of castor seeds. Several additional manuscripts are currently in preparation or in review that address these questions in detail.

Conclusions

The collection of *R. communis* seeds and seed-derived materials displayed a wide range of isotope ratios, as expected from the range of growth environments from which they were collected. The distributions of C, N, O, and H isotope ratios followed patterns predicted from previous studies and mass balance. The N content of the castor seed is virtually identical to that of protein extracted from the seed, whether the extract is a crude mixture or highly purified ricin; thus, N isotope ratios could be used to associate potential source seeds with a ricin sample, whether that sample was prepared simply by solvent extraction of seeds, as a crude protein precipitate, or highly purified. Over 99% of ricin samples prepared by each method could be distinguished from all other samples prepared by that method by comparing C, N, O, and H isotope ratios, demonstrating that these ratios represent a powerful forensic signature for sample matching and differentiation.

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