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Laboratories and Demonstrations

The Application of Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) to the Analysis of Alcoholic Beverages

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The distribution of deuterium in a molecule may be used as a chemical "tracer"….

The relative deuterium concentration and specific deuterium-site locations in a molecule can be determined using Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR). For a given compound (e.g., ethanol) SNIF-NMR can provide information about the chemical pathway of formation and, in some cases, information about the geographic origin of a sample can also be discerned. SNIF-NMR has been applied to the analysis of wines and other alcoholic beverages. In this work, data were collected on samples of apple brandy, tequila, rum, potato vodka, cognac, and synthetic ethanol. Signal-to-noise considerations limit the samples that can be studied without preconcentration to those with relatively high alcohol contents.

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NMR experiments are routinely performed in the undergraduate laboratory using ^{1}H and ^{13}C nuclei to obtain structural information about samples, Modern NMR instruments, however, can be used for many other applications. For example, NMR has been applied to variable temperature kinetics [1], multinuclear characterizations of organometallic complexes [2], titration of tripeptides [3], gas-phase studies of dynamic processes [4], and paramagnetic susceptibility of transition metal ions [5]. This work describes a quantitative NMR experiment designed to distinguish between ethanol samples from different sources based on the relative amounts of deuterium in the samples and the relative distribution of deuterium within the ethanol molecules.

The deuterium that occurs naturally as a small fraction of hydrogen isotopes is not distributed uniformly throughout the globe. Natural isotope distributions are the result of continuous chemical and physical processes. For example as water evaporates, more HDO remains in the liquid phase than does the more volatile H_2O. When water evaporates from warmer climates near the equator, the water vapor, enriched in H_2O, reaches the cooler regions of the earth and condenses back to liquid. The result is a low concentration of HDO in the polar regions relative to the concentration at the equator [6]. In fact the actual deuterium content in water ranges from approximately 90 ppm at the South Pole to about 160 ppm at the equator [7].

The abundance of deuterium in an organic compound is typically determined by mass spectrometry [8]. The analysis technique involves combustion followed by reduction of the water to produce H_2 gas. The isotopic content is then measured by mass spectrometry [9]. This method yields the overall deuterium content of a compound, but it fails to reveal the distribution of the deuterium within the compound. The distribution of deuterium in a molecule may be used as a chemical “tracer” in the analysis of many naturally occurring compounds, and may reveal the geographic and/or biological history of certain samples. This is not a new technique; in fact, shortly after Harold C. Urey discovered deuterium in 1932, it was found that variations in isotope distributions in fossil shells could provide information about climatic changes [10].

The deuterium distribution in a sugar molecule varies as a function of the specific photosynthetic pathway used in its synthesis, the HDO content of the water consumed by the plant, and the environmental conditions of the plant’s habitat. Plants in which the sugar is synthesized by the Hatch–Slack C_4 cycle distribute deuterium differently

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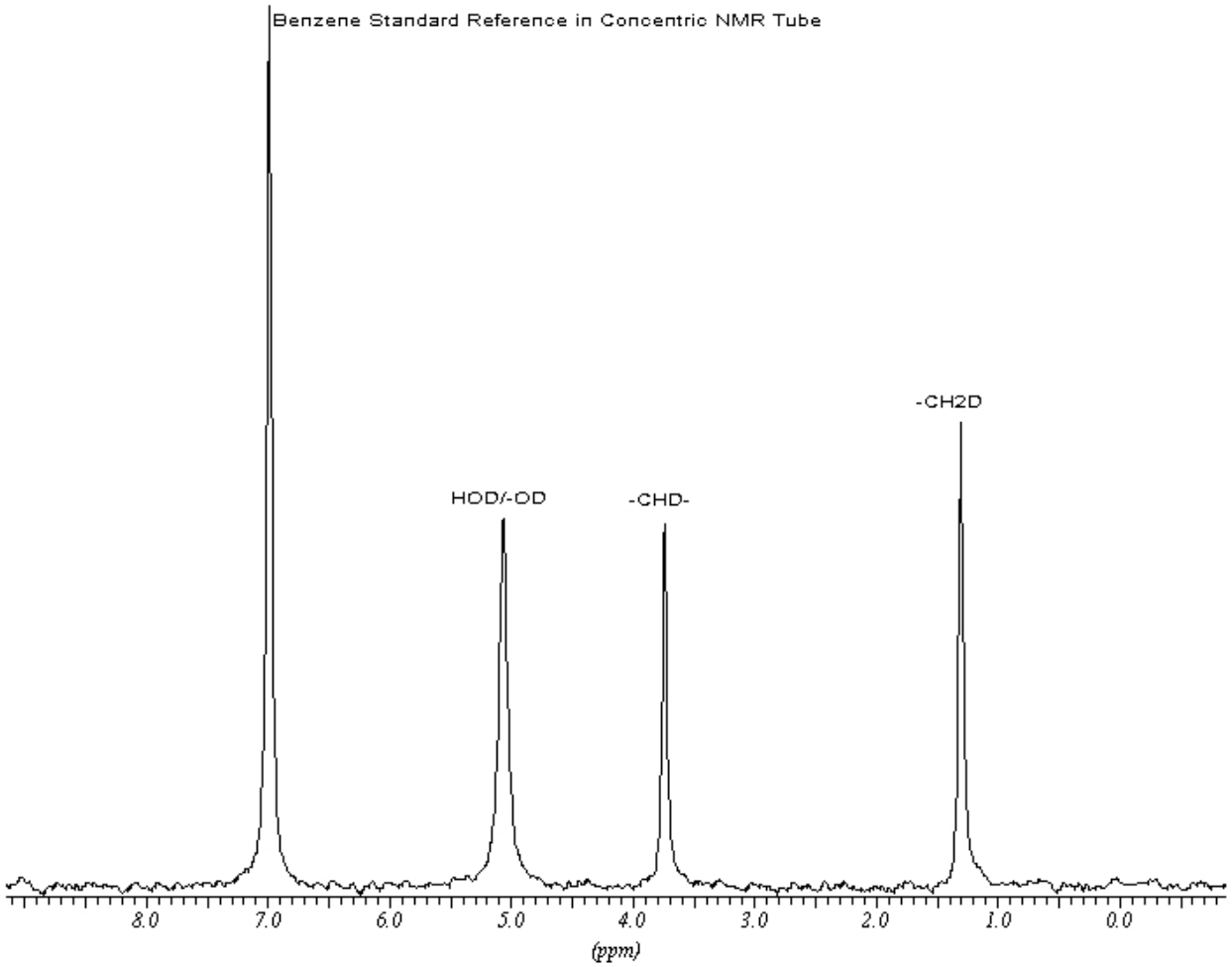

FIGURE 1. DEUTERIUM NMR SPECTRUM OF BACARDI RUM.

than do plants which utilize the Calvin C_3 cycle [9]. Obtaining a suitable deuterium NMR spectrum of sugar to observe this distribution is quite difficult, as the spectrum is complex and has many overlapping peaks [7]. When sugars are fermented, the isotope distribution of the resulting ethanol fortunately parallels the distribution of deuterium in the sugar molecule. Thus, deuterium NMR spectroscopy of the ethanol produced by the fermentation will produce a much-simplified spectrum while retaining at least some of the information regarding the source of the sugar (Figure 1).

In ethanol, deuterium may be located in three sites:

$HOCH_2CH_2D$	$HOCHDCH_3$	$DOCH_2CH_3$
(I)	(II)	(III)

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The deuterium NMR signals of HDO and the —OD group of ethanol overlap at the concentrations encountered in this experiment, therefore, species (III) will be ignored.

Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) [7], offers a method of analysis that can yield the isotopic abundance of deuterium, as well as its distribution, in ethanol. SNIF-NMR has been applied to the analysis of many different compounds, but has most frequently been used in the food industry. For example, Eurofins Scientific is an international group of laboratories that use SNIF-NMR for the analysis of wines and other alcoholic beverages. The technique is used to identify counterfeits, detect dilutions, and to check for adulterations such as the addition of sugar from different sources prior to fermentation [11].

Experimental

Most NMR users are very familiar with the need for good magnetic homogeneity in order to obtain sharp, well-resolved peaks. To obtain this, shimming is usually performed by optimizing the deuterium lock signal from the deuterated solvent in which the sample is dissolved. To obtain a ^{2}H-NMR spectrum, there are two choices: using a lock signal other than deuterium (i.e., ^{19}F, an expensive option), or running the spectrometer unlocked. Running unlocked requires shimming by using the FID of any nucleus present in the sample that yields a reasonable signal-to-noise ratio in one pulse. In the deuterium SNIF-NMR experiment, the obvious choice is to shim on the proton FID. This is accomplished by setting the acquisition parameters of the instrument to observe the single-pulse FID in a continuous mode. In this mode the spectrometer displays the FID and the integrated area from a single pulse, then erases those data and displays the result from the next pulse. As these pulses are acquired, the shims are adjusted by the operator to obtain the best FID shape and the greatest integrated area. Running the spectrometer unlocked opens the door for instrument drift. This is a significant concern in this experiment because lengthy (~2-h) acquisitions are required. The key to obtaining reproducible data is to achieve the greatest signal-to-noise ratio in as short a time as possible. Coupled with the difficulty of acquiring spectra in an unlocked mode is the necessity of obtaining good quantitative data. Therefore, adequate time must be allowed between each pulse for the nuclei to achieve complete relaxation. Typical suggestions range between 5 and 10 times the T_1 (relaxation time) of the slowest-relaxing nucleus. The deuteriums at sites I and II have T_1 values of ~0.95 s and ~1.05 s, respectively [12]. Accurate determination

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of the 90° pulsewidth is essential to obtaining good signal-to-noise ratios. Any deuterated solvent can be used to determine the 90° pulsewidth for deuterium.

$^{2}H\{^{1}H\}$ NMR spectra were recorded on a Bruker DRX-400 FT-NMR spectrometer at 61.38 MHz using a 5-mm broadband tunable probe. The deuterium spectra are acquired using WALTZ (composite pulse) decoupling. It has been shown that there is virtually no NOE effect on the spectra [12]. Spectra were acquired using a 90° pulse (13 μs), WALTZ decoupling, and a relaxation delay of 5 s. The spectral width was 919.66 Hz (~18 ppm) using 16 kB of data, resulting in an acquisition time of 6.685 s. All samples were run unlocked as neat liquids in a 5-mm-diameter coaxial tube (Wilmad WGS-5BL) at 297 K. A reference standard of 1 μL benzene-d_6 in 2 mL of benzene was contained in the sealed, inner coaxial tube. This standard capillary was used throughout all experiments. The probe was carefully tuned and the spectrometer was shimmed on the proton FID prior to each experiment. Data from 600 pulses were collected in each experiment. A line-broadening factor of 2.00 Hz was applied in the exponential apodization processing of the FIDs. Experiments were repeated a minimum of 10 times to determine reproducibility (Table 1). The acquisition time for a single experiment is approximately 2 hours.

The six samples chosen for this experiment have relatively high ethanol content: synthetic ethanol (Aaper Alcohol 200 proof), rum (Bacardi, 151 proof), tequila (Cuervo Especial, 80 proof), cognac (Remy Martin, 80 proof), brandy (Apple Jack, 100 proof), and vodka (Luksusowa, 80 proof). The synthetic ethanol was prepared by the direct catalytic hydration of ethylene, the rum was fermented from sugar cane, the tequila came from the agave plant, the cognac from grapes, the brandy from apples, and the vodka from potatoes. The analysis of 5 or 6 alcohols may best be conducted as a class project in which each group (2–4 students) is given a different sample to analyze. The students can then pool their data to produce a plot similar to Figure 2.

Results and Discussion

In SNIF-NMR experiments with ethanol, a defined parameter, R, is frequently used to describe the relative deuterium distribution in the ethyl fragment of the molecule [13].

$$R = \frac{3(II)}{(I)} \qquad (1)$$

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TABLE 1. Statistical data for the experimentally determined relative deuterium concentration in the alcohol fermented from various sugar sources.

Sample Source	No. of Experiments	Value	Average	Standard Deviation	Standard Deviation of the Mean
Synthetic	15	R	2.262	0.027	0.007
		C	1.464	0.010	0.003
Apple	20	R	2.552	0.032	0.007
		C	1.140	0.016	0.004
Grape	10	R	2.528	0.023	0.007
		C	1.173	0.012	0.004
Potato	15	R	2.697	0.043	0.011
		C	1.083	0.014	0.004
Sugar Cane	11	R	2.312	0.019	0.006
		C	1.232	0.012	0.004
Agave	20	R	2.235	0.027	0.001
		C	1.203	0.007	0.001

(I) and (II) represent the peak heights for the corresponding deuterium methyl (I) and methylene (II) signals relative to the height of the internal standard. If there was no isotope fractionation, and the deuterium was distributed statistically, the ratio of deuteration at site (I) to site (II) should be 3:2, which corresponds to an R value of 2.0. Values of R greater than 2 indicate deuterium enrichment in the methylene site (II) with respect to the methyl site (I) [9]. The value of R varies according to the fermentation process and the sugar used. Water present during the fermentation has a significant effect on the deuterium content of the methylene site, while the sugar determines the isotope concentration at the methyl site [10].

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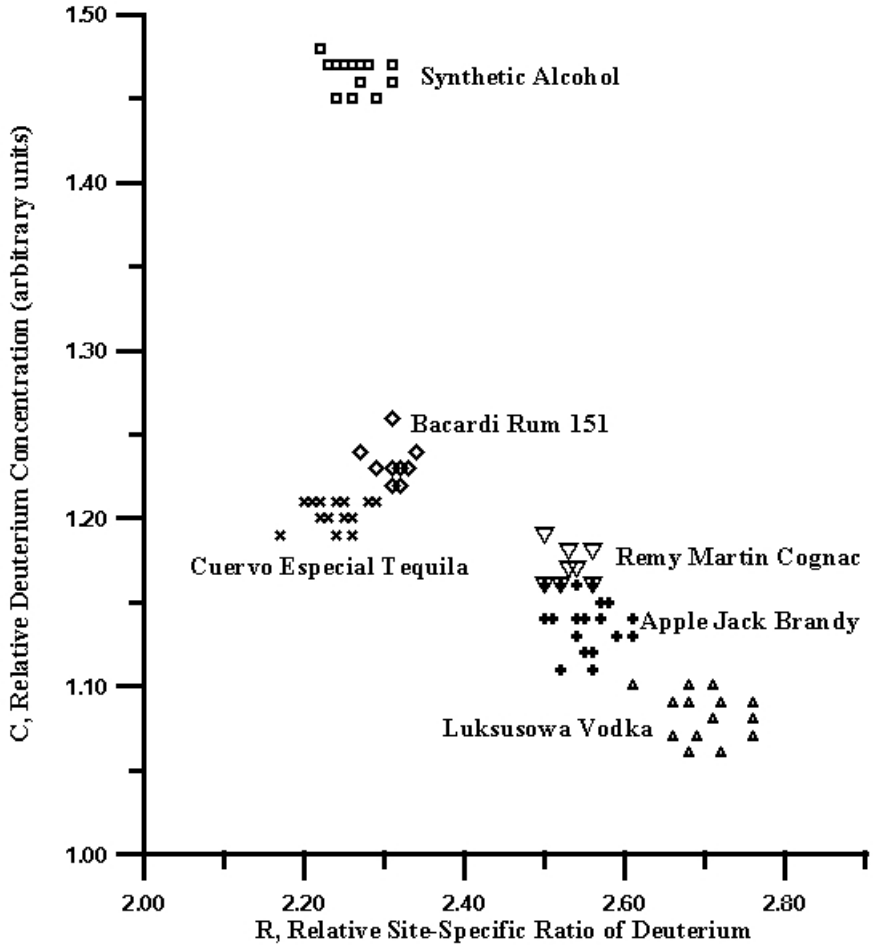

FIGURE 2. DEUTERIUM IN ETHANOL.

The R value alone is adequate for discerning some, but not all, sugar sources. The relative deuterium concentration, C, can be used to provide additional information about samples.

$$C = \frac{(I)+(II)}{(s)\kappa} \tag{2}$$

(I) and (II) are the peak heights of the methyl and methylene sites respectively; (s) is the peak height of the external standard. To account for the fact that not all samples have equal ethanol concentrations, a correction factor, κ , must be employed. For example, a sample of pure ethanol would have a correction factor of 1.00, while a 40%

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ethanol sample would have a correction factor of 0.40. (Government regulations require that the proof listed on the label be accurate within 1%.)

Ethanol samples from the same source (i.e., sugar cane grown at one location) should have similar R and C values. Ethanol from different sources (e.g., sugar cane vs potato) or different locations (e.g., Germany vs France) should have R and C values that can be readily discerned from one another. A plot of the relative site-specific ratios of deuterium, R, versus the relative deuterium concentration, C, for the previously listed samples is illustrated in Figure 2.

After obtaining the spectra, the peak heights of the methyl (I) and methylene (II) sites were measured relative to the standard. Assigning the height of the standard peak a value of 1.00 scales the C values to between 1.00 and 1.50. Multiple experiments on the same sample provide a cluster of data points in the plot of the relative site-specific ratio of deuterium, R, on one axis and the deuterium concentration relative to the standard, C, on the other (Figure 2). The scatter associated with this data is too large to distinguish between different geographic sources of sugars. However, even though some individual data points for different samples (i.e., cognac from grapes vs brandy from apples) overlap, the mean values are distinguishable. The instrumental requirements (a 15-mm ^{2}H–^{1}H probe and a 600-MHz [^{1}H] NMR spectrometer) that would permit geographic distinctions are beyond the capabilities of most undergraduate laboratories.

The alcohol concentrations in the selected samples are high enough (≥ 40%) so that preconcentration is not required. Samples with lower concentrations of alcohol, such as wines, would require careful distillations and determination of the alcohol concentration.

Conclusion

Deuterium is a nucleus that heretofore has not been included in the repertoire of NMR experiments performed in the undergraduate laboratory. Running the spectrometer unlocked and learning how to shim on the FID offers students additional insights into how the spectrometer works. In addition, this experiment illustrates the capability of NMR for quantitative applications.

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