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TECHNICAL NOTE

Mark A. Kirms,[1] Ph.D. and Lynn M. Kirms,[2] Ph.D.

The Identification of Sodium Fluoroacetate (Compound 1080) Employing NMR Spectroscopy

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ABSTRACT: Nuclear magnetic resonance (NMR) spectroscopy was employed for the purpose of identifying samples of materials suspected of containing sodium fluoroacetate (Compound 1080). Acquisition of routine proton (^{1}H) and carbon (^{13}C) NMR spectra provided a straight-forward means for determining the presence of Compound 1080 in the samples and thus afforded a simple method for analysis and identification of this compound.

KEYWORDS: forensic science, NMR, sodium fluoroacetate, compound 1080, 1080, fluoroacetic acid, spectroscopic analysis

The capacity of nuclear magnetic resonance (NMR) spectroscopy to elucidate molecular structures and to solve chemical problems is unparalleled in the field of analytical methodology. This can be seen in the use of NMR spectroscopy to elucidate protein and DNA structure, structures of polymeric materials, or the enantiomeric purity of pharmaceuticals (1). While NMR is embraced by the greater chemical community as a powerful method to solve a wide array of chemical problems, and has made strong inroads into the medical community as a diagnostic tool via magnetic resonance imaging (2), the full potential of NMR for solving forensic problems has yet to be realized. The past decade has witnessed only a smattering of articles appearing in the forensic science literature which center on the use of NMR spectoscopy for forensic applications, and these are primarily in the area of illicit drug identification (3–9).

We have had the opportunity to apply NMR spectroscopy to the problem of identifying samples thought to be sodium fluoroacetate ($F_3CCOO^-Na^+$). Sodium fluoroacetate, also known as Compound 1080 or 1080, is a toxin which acts to disrupt cellular respiration through the formation of fluorocitrate, leading to respiratory failure and cardiac arrest. It is a compound which is highly toxic to a variety of wildlife and, as such, is a restricted use chemical. Currently, the only legal application of 1080 is for the use of toxic livestock collars to control loss of livestock from predation. Structurally, sodium fluoroacetate is a simple molecule. While simple in structure, however, determining the presence of the molecule in various types of samples may be deceptively challenging. Several methods for determining the presence of 1080 in a sample require derivatization of the molecule to a suitable ester functionality for detection by gas chromatography (GC) coupled with an electron capture detector or mass selective detector (10,11) or high performance liquid chromatography (12–14). One direct means of analysis of sodium fluoroacetate without derivatization is by ion selective electrode (fluoride) (15). While many of these methods of detection can be applied to the analysis of 1080 at low concentrations in a particular sample, the analyses require "wet chemistry" to be performed on the sample and consequently make such analyses cumbersome and often time consuming. In contrast, NMR spectroscopy can readily provide a direct analysis when larger quantities (about 1 mg) of the material to be analyzed are available (Spectra may be obtained on sample sizes as small as 1 μg with the appropriate instrumentation) (16). In considering NMR spectroscopy as a method of analysis for sodium fluoroacetate, one possible methodology is direct study by ^{19}F NMR. Fluorine NMR spectroscopy has been used to analyze for fluoroacetate in plants and prepared bait samples (17–19). While fluoroacetate can be easily identified by ^{19}F NMR, it does require the use of a special probe. Standard to all NMR instruments, however, are ^{1}H and ^{13}C capabilities. One can indirectly show the presence of a fluorine atom without performing ^{19}F NMR through NMR experiments involving these two standard nuclei. We sought to take advantage of the ease and the powerful structural elucidation capabilities of NMR analysis with instrumentation that is broadly available to identify compound 1080 by ^{1}H and ^{13}C NMR spectroscopy.

Materials and Methods

The NMR work was performed on a JEOL CPF 270 MHz NMR spectrometer equipped with variable temperature capabilities. Deuterium oxide (D_2O) containing 1%w/w of the sodium salt of 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) as an internal reference was purchased from Aldrich Chemical Company. An alternative solvent system is D_2O containing 0.75% w/w of the sodium salt of 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, also available from Aldrich Chemical Company. Sodium Fluoroacetate used as standards was obtained from Aldrich Chemical Company and the Environmental Protection Agency (EPA). The samples studied were from cases under investigation by the National Fish and Wildlife Forensics Lab and were received as either liquids or solids. Solid samples were used directly by dissolving 10–20 mg of

[1]National Fish and Wildlife Forensics Laboratory, 1490 East Main Street, Ashland, Oregon.
[2]Chemistry Department, Southern Oregon University, Ashland, Oregon.
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sample in 1.5mL D_2O containing DSS. Undissolved solid was filtered through a Pasteur pipette fitted with a cotton plug. Samples that were liquids were heated in an open atmosphere or under water pump vacuum to remove the solvent. The remaining residue was then treated in a similar manner as the solid samples. All ^1H NMR spectra were recorded at 270.05 MHz employing a spectral width of 5405.4 Hz (−1.0 to 19.0 ppm), a 3.0 second pulse delay, and a 5.7 μsec pulse width. Single Pulse with Broadband Decoupling ^{13}C NMR spectra were recorded at 67.80 MHz with an 18,050 Hz spectral width (−24 to 242 ppm), a 5,400 Hz irradiation frequency, a 3.0 μsec pulse width, and a 1.00 sec pulse delay. Distortionless Enhanced Polarization Transfer (DEPT) spectra were collected using the DEPTEDIT2 program (provided with the instrument software) run at 67.80 MHz with an 18,050 Hz spectral width (−24 to 242 ppm), a 5,400 Hz irradiation frequency, an 8.7 μsec pulse width, and a 2.5 sec pulse delay.

Results and Discussion

The examination of the ^1H and ^{13}C spectra of sodium fluoroacetate arose out of a study of two samples sent to the National Fish and Wildlife Forensics Laboratory for analysis. One sample arrived as a purple colored liquid, the other as an off-white solid. Running ^1H and ^{13}C NMR spectra to confirm the identities of these samples was an attractive option given that an NMR spectrometer was readily available. It was expected that manipulation of the samples would be minimal, and that the analyses could be done very quickly. Indeed, ^1H and ^{13}C spectra turned out to provide a facile and uncompromisingly clear identification of Compound 1080.

The unfluorinated analog of sodium fluoroacetate is sodium acetate. Its ^1H NMR spectrum consists of a lone singlet at 1.9 ppm, which is indicative of the methyl group adjacent to the carbonyl. The effect of substitution of a fluorine atom in place of one of the hydrogens on the methyl group of sodium acetate should be quite obvious in the ^1H NMR spectrum of sodium fluoroacetate. In running NMR spectra of the lab samples suspected of being sodium fluoroacetate, it was expected that the presence of fluorine would be indicated by a significant downfield chemical shift of the hydrogens of the $—CH_2F$ group, and, because ^{19}F and ^1H can undergo geminal coupling, one should observe a doublet for the $—CH_2F$ system of the fluoroacetate. Indeed, this is what was ultimately observed. Figure 1 is the ^1H NMR spectrum of one of the lab samples received as a liquid for analysis. The initial attempt at taking the spectrum yielded only a large H_2O signal, which confirmed the nature of the solvent that the sample was dissolved in. Removal of excess water yielded a slightly more useful spectrum, albeit, not a conclusive one. Water still remained in the sample and was responsible for the broad signal observed at 4.8 ppm. The small signal at 4.61 ppm is one half of a doublet centered at 4.70 ppm; the other half of the doublet being obscured by the water signal. Such a doublet at 4.70 ppm fits the characteristics of the CH_2F group of fluoroacetate in regards to chemical shift and multiplicity. To verify the presence of the doublet, a very simple variable temperature NMR experiment was done, the results of which are seen in Fig. 2. The same sample was heated to 60°C, and the ^1H NMR spectrum re-taken. Changes in hydrogen bonding characteristics at higher temperatures caused a shift of the water signal to a more upfield position, thus affording a clear confirmation of the presence of the anticipated $—CH_2F$ doublet. The equivalent integration signals of the peaks at 4.78 and 4.63 ppm lend credence to this assertion. The presence of the fluorine in the molecule was also supported through the ^1H —^{19}F coupling that was observed. Geminal $^{19}F—^1H$ coupling constants are notably large, and so, the 48 Hz $^{19}F—^1H$ coupling constant that was found for the $—CH_2F$ doublet was in accord with our expectations (20). The ^1H NMR spectra taken of this lab sample were in complete agreement with those taken of a 1080 sample obtained from the EPA.

The ^{13}C proton decoupled NMR spectra of sodium fluoroacetate

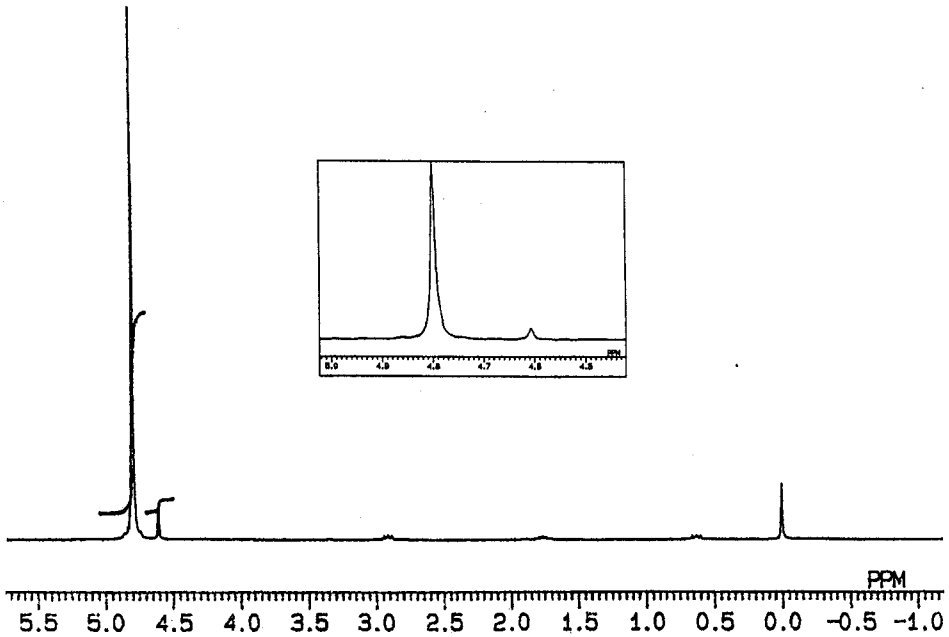

FIG. 1—*Room temperature ^1H NMR spectrum of liquid sample suspected to be 1080.*

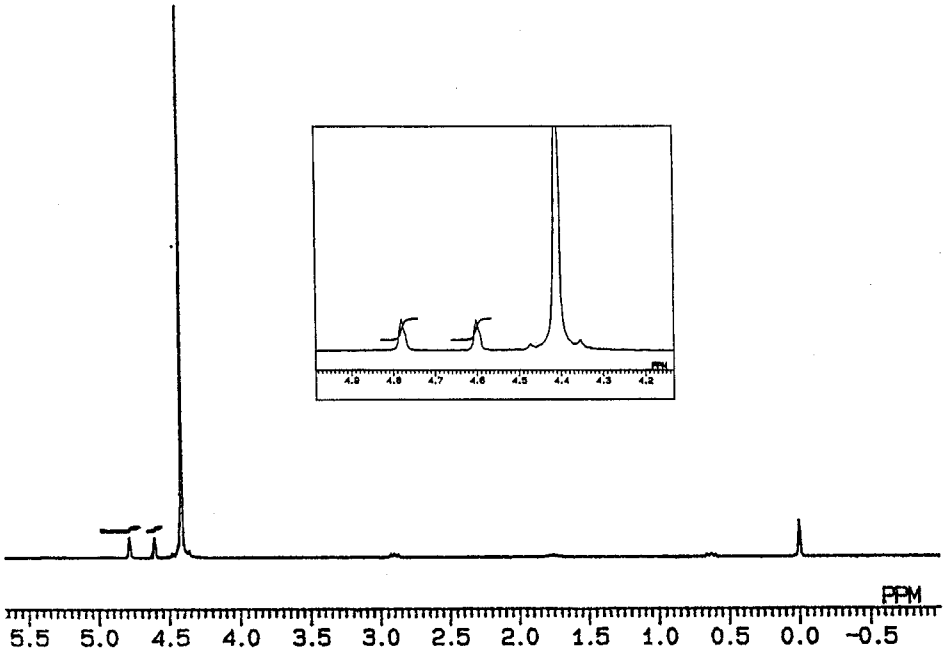

FIG. 2—*Variable temperature ^{1}H NMR spectrum of liquid sample taken at 60°C.*

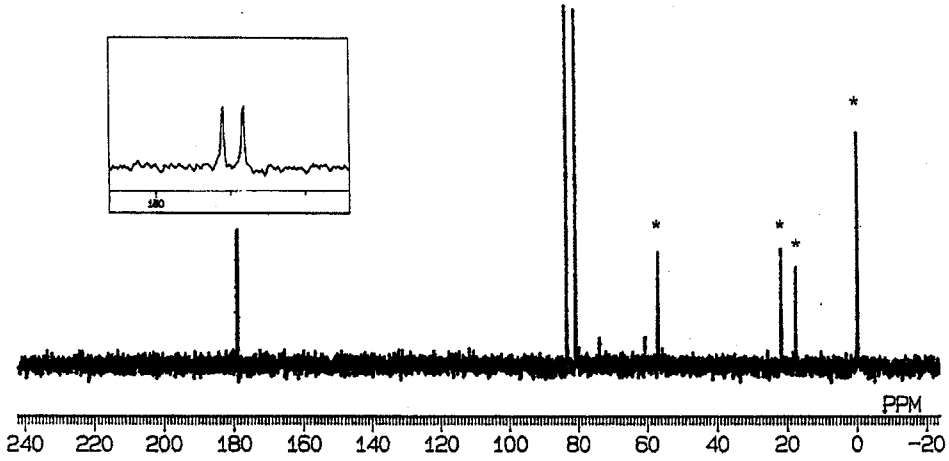

FIG. 3—*^{13}C NMR spectrum of solid sample suspected to be 1080.*

also provides an undeniable signature for the presence of fluorine through ^{13}C—^{19}F coupling (Fig. 3). This particular spectrum was taken of an off-white solid purported to be compound 1080. Only two carbon signals were expected for the fluoroacetate and this is what was observed. The spectrum afforded a signal for the carbonyl carbon centered at 178.9 ppm, and a signal for the carbon of the —CH_2F group, centered at 82.1 ppm (The other peaks marked with asterisks are due to the internal standard). The signal for the —CH_2F group showed characteristically strong one bond ^{19}F—^{13}C coupling. Since fluorine also exhibits unusually strong long range coupling, the carbonyl carbon signal appeared as a doublet (see inset), a result of the splitting of the carbonyl carbon by fluorine. These ^{13}C NMR spectra matched those taken of the 1080 standard obtained from the EPA.

A quick and easy ^{13}C experiment to perform that further associates the hydrogens and carbon atoms in a molecule is a DEPT experiment. Normally, ^{13}C decoupled proton spectra do not afford any information as to the number of hydrogens present on each carbon, but in a DEPT experiment, methine, methylene, and methyl groups exhibit different phases and can be differentiated. Figure 4 shows the results of a DEPT experiment run on one of the lab samples suspected of being 1080. (The peaks marked with an asterisk are due to the internal standard.) The standard, proton decoupled ^{13}C spectrum of the lab sample is shown in the bottom trace. The other three traces are a result of the DEPT experiment and differentiate between methyl, methylene and methine groups. Since the carbonyl carbon bears no hydrogens it does not appear in any of the traces. However, the signal at 80 ppm is clearly identified as a CH_2

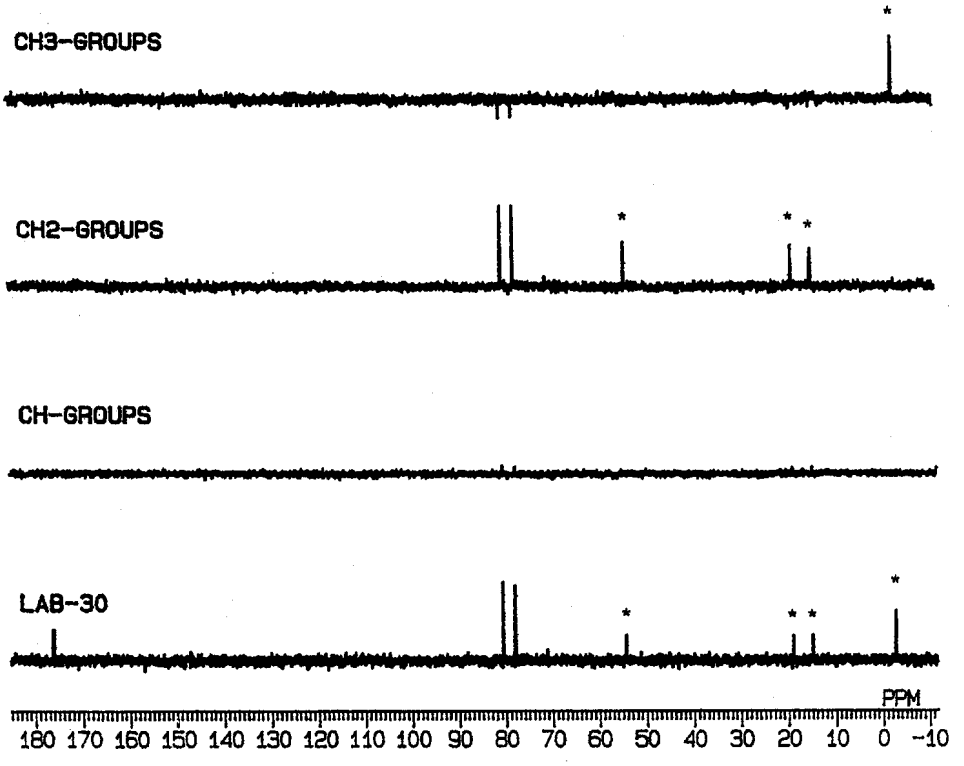

FIG. 4—*Results of DEPT experiment carried out on solid sample.*

group in the second trace further establishing the presence of the —CH_2F group in the molecule.

Conclusions

The use of ^1H and ^{13}C NMR spectroscopy is a powerful and efficient method for analysis of the presence of sodium fluoroacetate. The power of NMR to potentially elucidate structure such that each *atom* of a molecule is unequivocally identified puts it in the category of a standalone technique. Its efficiency lies in the fact that it is a non-destructive method that does not require any derivatization steps: once a sample has been made up, much information regarding the identity of an unknown can be obtained by multiple NMR experiments, (which are quickly interchanged by mere keystrokes at a computer) obviating the need for *any* manipulation in the laboratory. Such an analysis can be easily completed in a very short time frame. Considering these factors and the increased availability and use of NMR spectrometers in government and academic research labs, one has in hand an extremely attractive method for the identification of compounds such as 1080.

Acknowledgments

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Additional information and reprint requests:
Mark A. Kirms, Ph.D.
National Fish and Wildlife Forensics Laboratory
1490 East Main Street
Ashland, Oregon 97520