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# A New Method for the Analysis of Granular Pesticide Formulations

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A new method is described for examining the chemical nature of a pesticide as a granular formulation. A screening program revealed that β-nitrostyrene was highly active against soybean root knot nematode but a granular formulation was completely inactive. Solid-state (CPMAS) ^{13}C NMR spectroscopy of a granular formulation prepared with ^{13}C-labeled active ingredient led to identification of the inactive form as a polymer of the active ingredient. Initiation of polymerization by the clay granule carrier was confirmed.

β-Nitrostyrene (1) was shown to be highly effective in the control of soybean root knot nematode and soybean cyst nematode in preliminary greenhouse tests when applied as a soil drench in acetone–water solution. In preparation for field testing, a granular formulation of 1 was prepared and applied in a greenhouse test. No control of nematode infection could be detected at any of the test rates with the granular formulation. Furthermore, no appreciable amount of 1 could be extracted from the granular formulation.

## EXPERIMENTAL SECTION

Labeled starting materials were purchased from MSD Isotopes and were of 99% isotopic purity at the labeled carbon. Solution NMR spectra were obtained in chloroform-*d* on a Bruker WM-360 spectrometer (^{13}C resonance frequence 90.6 MHz) operating in the pulsed Fourier transform mode. Chemical shifts are reported (ppm) downfield from the internal standard tetramethylsilane. Infrared spectra were obtained on a Perkin-Elmer Model 781 infrared spectrometer as solid dispersions in potassium bromide.
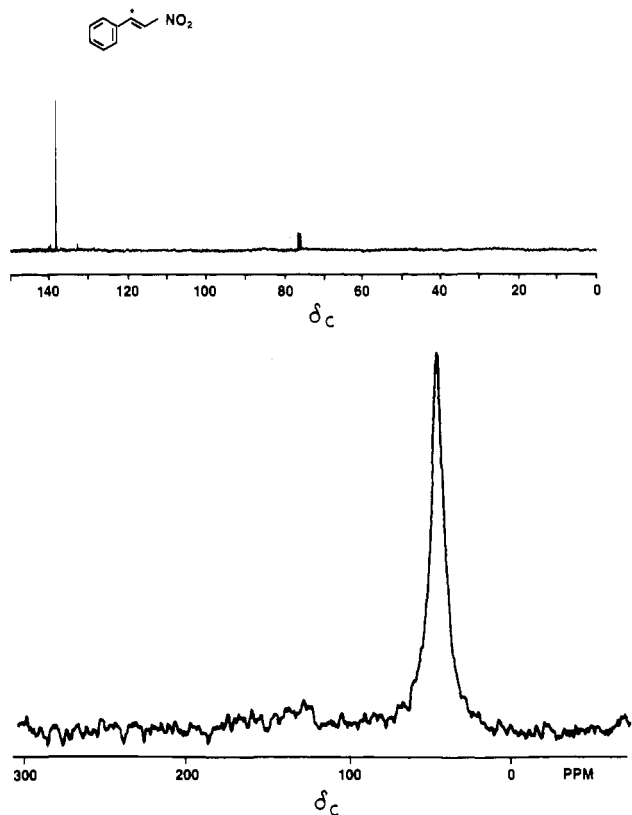
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**Solid-State NMR.** Cross-polarization magic-angle spinning (CPMAS) (Schaefer and Stejskal, 1976) ^{13}C NMR spectra were collected on a home-built spectrometer operating at a proton Larmor frequency of 60 MHz corresponding to a ^{13}C resonance frequency of 15.1 MHz. Samples were spun at the magic angle (54.7°) with respect to the static magnetic field at a spinning speed of 1859 Hz. The spinner on the magic-angle probe had an internal volume of 600 μL, and a typical sample weighed 400–450 mg. A more detailed description of the magic-angle spinner can be found elsewhere (Schaefer et al., 1987). Spectra were collected following 500-μs matched [13]C–[1]H spin-lock contacts with high-power proton dipolar decoupling. The repetition rate on the experiments was 500 ms.

**Dipolar Rotational Spin-Echo ^{13}C NMR.** Dipolar rotational spin-echo (DRSE) ^{13}C NMR is a two-dimensional NMR technique that allows the strength of [13]C–[1]H dipolar interactions to be measured (Munowitz and Griffin, 1982; Schaefer et al., 1983). With [1]H–[1]H couplings suppressed by homonuclear multiple-pulse decoupling (Waugh et al., 1968; Mansfield et al., 1973; Rhim et al., 1973; Burum et al., 1981), magic-angle spinning caused the [13]C–[1]H dipolar line shape associated with a particular carbon spin to break up into spinning sidebands. Because the characteristic dipolar spinning-sideband patterns for [13]CH and [13]CH$_2$ groups are quite different, the experiment can be used to count the number of protons attached to a particular carbon (Webb and Zilm, 1989).

DRSE ^{13}C NMR experiments were performed on the same

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**Figure 1.** $^{13}$C NMR of [$\alpha$-$^{13}$C]$\beta$-nitrostyrene: (top) solution spectrum; (bottom) CPMAS spectrum of granular formulation. Sample weight 454 mg, 21 500 transients.

home-built spectrometer described above. Proton–proton multipulse decoupling was accomplished with use of a semiwindowless MREV-8 sequence (Rhim et al., 1973; Burum et al., 1981) with a cycle time of 33.6 $\mu$s. Carbon dipolar tensor simulations were performed by the methods developed by Herzfeld and Berger (1980).

**Preparation of Labeled 1.** [$\alpha$-$^{13}$C]-1 was prepared from [*carbonyl*-$^{13}$C]benzaldehyde, and [$\beta$-$^{13}$C]-1 was prepared from [$^{13}$C]nitromethane by the literature method (Worrall, 1941).

**Preparation of Granular Formulations.** In a typical procedure, 50 mg of labeled 1 was dissolved in 1 mL of acetone. This solution was added to 1.0 g of attapulgite clay granules, LBM 24/48, in a round-bottom flask. The flask was oscillated for 1 h, whereupon the wet granules were placed on an open dish and allowed to stand in the hood overnight. Samples for solid-state NMR experiments were ground to a fine powder with an agate mortar and pestle.

A 100-g sample of granular formulation prepared in the same manner but using unlabeled 1 was extracted in a Soxhlet extractor for 24 h with chloroform. Upon evaporation of the chloroform, 0.184 g of residue was recovered. Gas chromatographic analysis showed no peaks corresponding to 1.

**Polymerization of 1 by Clay.** A solution of 3.73 g of 1 in 100 mL of ethanol was stirred with 0.1 g of attapulgite clay granules for 4 days at room temperature. The solution became cloudy during this time. The mixture was filtered, the tan solid washed with three 25-mL portions of methanol, suspended in water, and centrifuged, and the lighter-colored solid at the top of the water layer removed and dried in air.

RESULTS AND DISCUSSION

It was thought that 1 either had evaporated during preparation of the granular formulation or had been chemically altered. In order to test these hypotheses, a sample of [$\alpha$-$^{13}$C]$\beta$-nitrostyrene was synthesized from [*carbonyl*-$^{13}$C]benzaldehyde by the standard method (Worrall, 1941). The solution $^{13}$C NMR spectrum of 1 is shown in Figure 1 (top). A granular formulation of this material
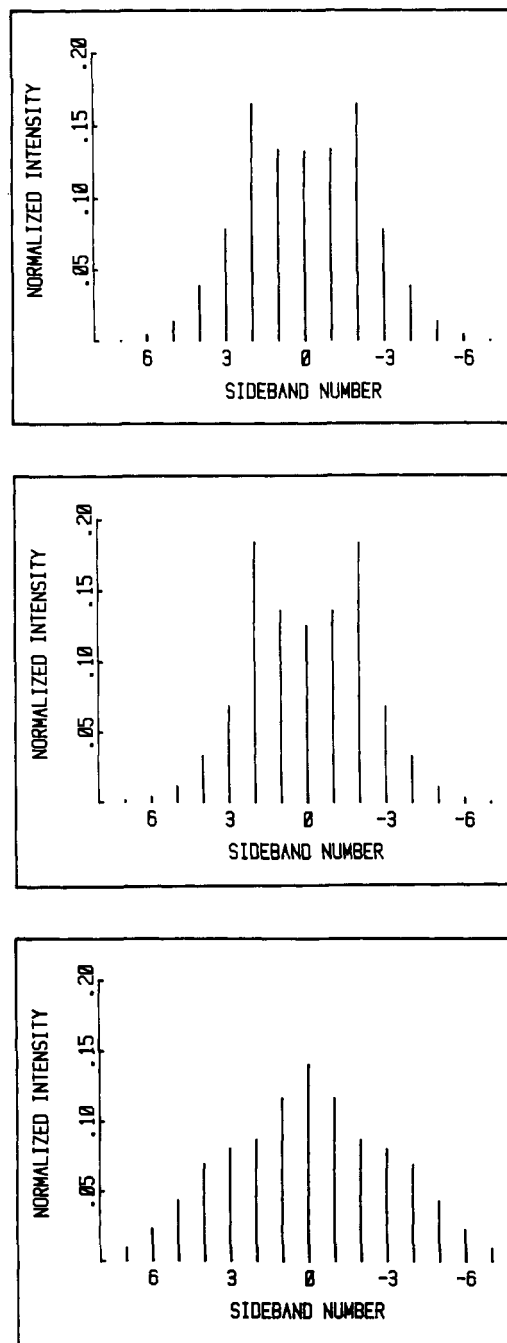


**Figure 2.** Dipolar rotational spin-echo $^{13}$C NMR: (top) experimental sideband pattern for granular formulation of [$\alpha$-$^{13}$C]$\beta$-nitrostyrene; (middle) calculated $^{13}$C–H sideband pattern; (bottom) calculated $^{13}$CH$_2$ sideband pattern.
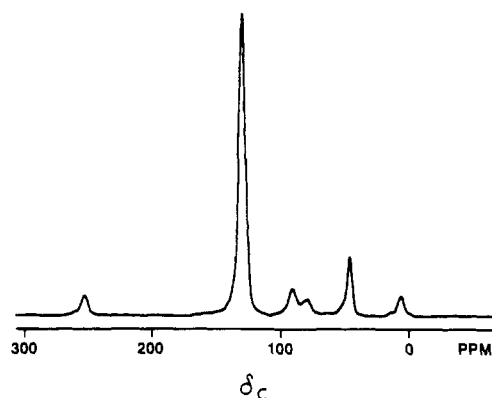


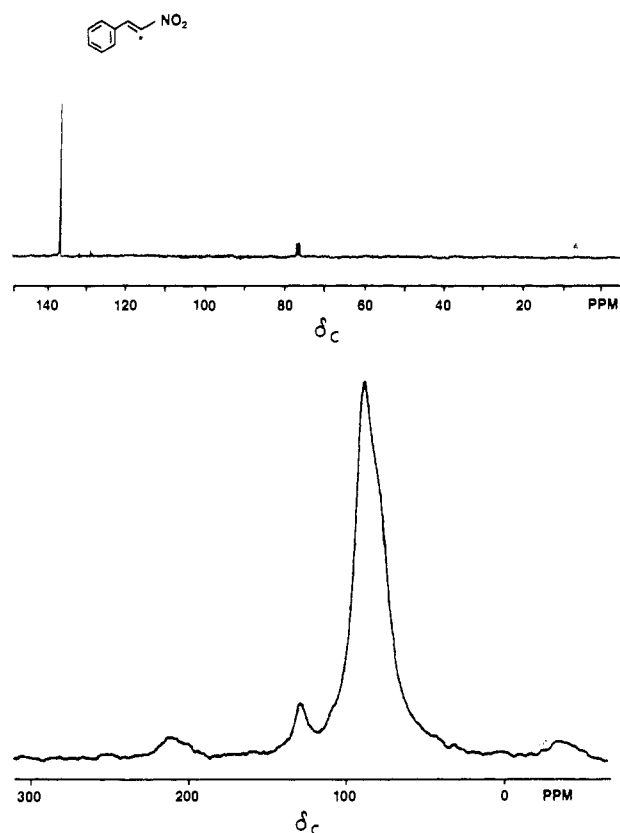**Figure 3.** CPMAS $^{13}$C NMR spectrum of $\beta$-nitrostyrene homopolymer. Sample weight 410 mg; 10 000 transients.

containing 5% active ingredient by weight was pre-
pared. The cross-polarization magic-angle spinning $^{13}$C
NMR spectrum of this solid material is shown in Figure
1 (bottom). This spectrum is dominated by a 48 ppm
signal due to the $^{13}$C label in 1, though natural-
abundance carbon signals from 1 are also visible. Dipo-
lar rotational spin-echo $^{13}$C NMR (see the Experimental
Section) demonstrates that the carbon giving rise to the
resonance at 48 ppm in this spectrum has one attached
proton (Figure 2).
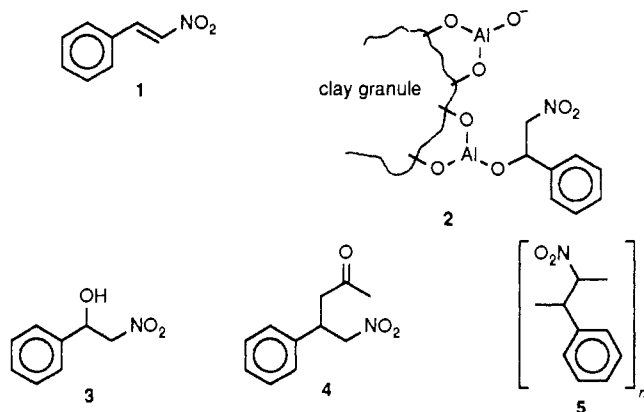


It was apparent from the spectrum of the granular for-
mulation that 1 had been altered radically; the chemical
shift of 48 ppm for the enriched carbon was consistent
with an alkyl rather than an alkenyl CH carbon. The
possibility that 1 had undergone Michael addition with
an oxygen anion either on the surface of the clay to give
a structure such as 2 or with water to give 3 was rejected
on the basis of the solution $^{13}$C NMR spectrum of 3 (Holle-
man, 1904). The α-carbon of 3 gives rise to a signal at
81.06 ppm, much further downfield than was observed
in the solid sample. The possibility that a base-
catalyzed addition of the acetone used as the solvent in
preparing the granular formulation had occurred to give
4 (Walker, 1965) was considered, since 4 does have a $^{13}$C
NMR signal at 45.7 ppm. This hypothesis was rejected
when a granular formulation prepared with tetrahy-
drofuran as the solvent showed an identical CPMAS $^{13}$C
NMR spectrum.

A third hypothesis, that the clay had catalyzed an anionic
polymerization of the olefin to give the homopolymer 5,
was subsequently confirmed. A sample of 5 was pre-
pared by polymerization of a solution of 1 with sodium
methoxide as an initiator (Carter et al., 1978). The nat-
ural-abundance CPMAS $^{13}$C NMR spectrum of this mate-
rial is shown in Figure 3. The α-carbon resonance appears
at 47 ppm while the β-carbon resonance, centered at 90
ppm, is a doublet due to dipolar coupling to $^{14}$N. This
latter assignment was confirmed by preparing β-$^{13}$C-la-
beled 1 from [$^{13}$C]nitromethane. A granular formula-
tion prepared from this material showed a large, broad
peak at 90 ppm in the spectrum shown in Figure 4 (bot-
tom).

That the clay was indeed initiating the polymeriza-
tion was confirmed by stirring an ethanol solution of 1
with clay granules for several days; an insoluble solid slowly
formed. The infrared spectrum of this solid, although
partly obscured by absorption from clay particles, shown
in Figure 5 (top), was clearly the same as that of the poly-
mer prepared by alkoxide-promoted polymerization, shown
in Figure 5 (bottom).



Figure 4. $^{13}$C NMR of [β-$^{13}$C]β-nitrostyrene: (top) solution
spectrum; (bottom) CPMAS spectrum of granular formulation.
Sample weight 375 mg; 87 000 transients.
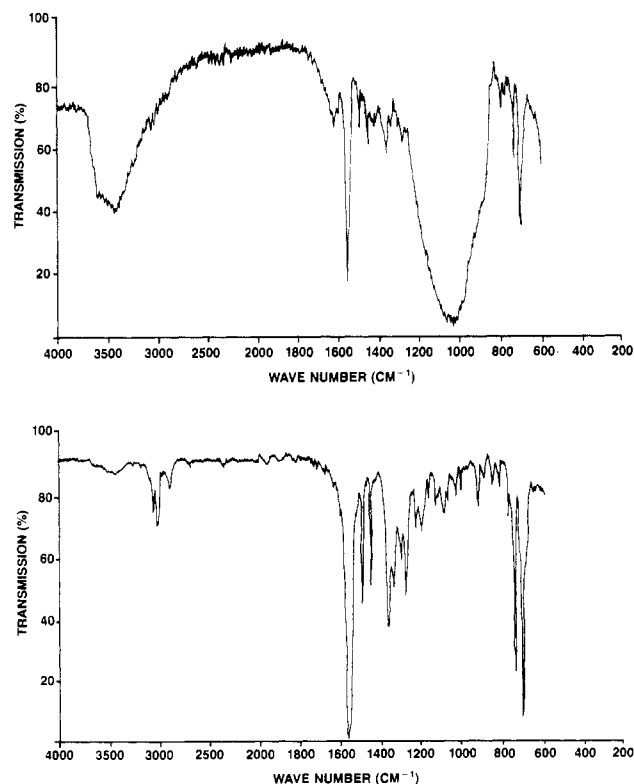


Figure 5. Infrared spectra of β-nitrostyrene homopolymer: (top)
from clay-catalyzed solution polymerization; (bottom) from alkox-
ide-catalyzed solution polymerization.

CONCLUSIONS

The combination of $^{13}$C chemical shift and attached-
proton data for β-nitrostyrene adsorbed on clay granules
showed that the compound had polymerized in the pres-

ence of the clay. This led to substitution of less reactive granular carriers, which gave biologically active granular formulations.

The application of CPMAS $^{13}$C NMR to the study of labeled materials as granular formulations shows great promise for the characterization of such formulations. Our experience with 1 indicates that adsorption onto granules does not induce large chemical shift changes; the chemical shifts observed in the solution NMR spectra of 1 are similar to those seen in the solid state. The use of $^{13}$C-labeled compounds involves no radioactivity, uses relatively cheap starting materials, and does not require large samples. (Each spectrum is obtained with about 20 mg of labeled active ingredient, and excellent signal to noise ratios can be obtained with overnight signal accumulation.) The state of the active ingredient on the granular carrier can be examined in a form typical for agricultural use, and delicate molecules need not be subjected to extraction or combustion analysis. In this work, qualitative information about the β-nitrostyrene/clay formulation was obtained from CPMAS $^{13}$C NMR spectra. Because intensities in these spectra are easily and reliably quantitated, the technique should find wide application in more quantitative studies of granular formulations as well.

## ACKNOWLEDGMENT

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# Stirring Continuous Extraction of Aqueous Organic Compounds with Fluororesin as a Water/Organic Solvent Separator

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A stirring continuous-extraction apparatus equipped with a fluororesin in a water/extracting solvent separation chamber was designed to isolate solvent-extractable material from foods and beverages rapidly and to destruct the forming emulsion. The new apparatus was evaluated by experiments with dilute aqueous solution of several model mixtures of volatile compounds. A comparison study of the extraction efficiency has been made for the following three methods: stirring continuous, continuous, and batch extraction. The stirring continuous extraction has equivalent or better extraction efficiency than the other extraction methods for all the compounds tested. Most of the compounds showed nearly quantitative extraction after 2 h. This method has been successfully applied to the separation of flavors from wine.

Liquid–liquid extraction (batch or continuous) offers a very simple and efficient means of isolating flavors from foods (Wilson et al., 1985; Engel et al., 1988) and beverages (Shimizu and Watanabe, 1982). The batch method is very simple but labor-intensive. Vigorous shaking for a considerable time is required to make the method effec-