Analysis of a Phenyl Ether Herbicide–Cyclodextrin Inclusion Complex by CPMAS ^{13}C NMR

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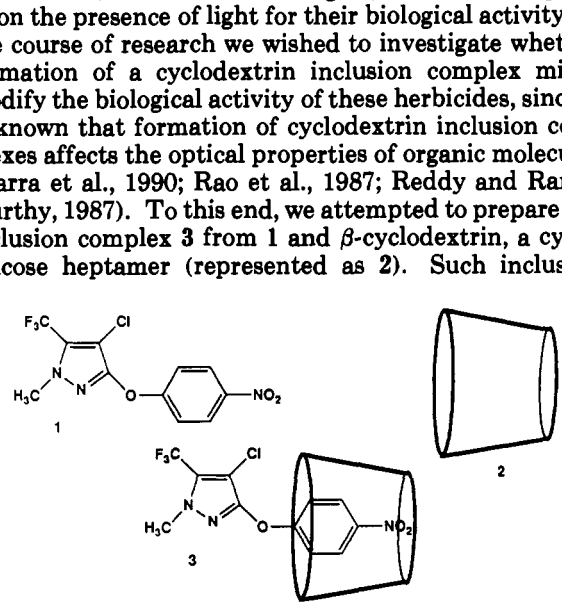
An inclusion complex of a pyrazole phenyl ether herbicide and β-cyclodextrin was prepared to investigate the effect of complex formation on herbicide activity. The usual methods of characterization (elemental analysis, X-ray powder diffraction, solution NMR) failed to show unequivocally that a 1:1 inclusion complex had formed. Analysis of relaxation times in CPMAS ^{13}C NMR spectra of the solid complex demonstrated that it was a true inclusion complex.

INTRODUCTION

Pyrazole phenyl ethers such as 1 are novel, highly active herbicides (Moedritzer and Rogers, 1990) that depend upon the presence of light for their biological activity. In the course of research we wished to investigate whether formation of a cyclodextrin inclusion complex might modify the biological activity of these herbicides, since it is known that formation of cyclodextrin inclusion complexes affects the optical properties of organic molecules (Barra et al., 1990; Rao et al., 1987; Reddy and Ramamurthy, 1987). To this end, we attempted to prepare the inclusion complex 3 from 1 and β-cyclodextrin, a cyclic glucose heptamer (represented as 2). Such inclusion



complexes are well-known (Szejtli, 1984) and have been characterized by a variety of physical techniques, including X-ray powder diffraction and NMR spectroscopy. It has been shown that the X-ray powder diffraction pattern of a solid isolated after complex formation is different from that of the starting cyclodextrin, and this difference has been used to argue that an inclusion complex has been formed (Rao et al., 1987). A number of solid-state ^{13}C and ^{2}H NMR studies of cyclodextrin inclusion complexes have been reported whose primary focus has been the measurement of the conformation and/or molecular dynamics of the small "included" molecules (Ronemus et al., 1988; Hall and Lim, 1986; Inoue et al., 1983; Saito et al., 1982; Ueda and Nagai, 1981).

EXPERIMENTAL PROCEDURES

Cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR spectra (Schaefer and Stejskal, 1976; Mehring, 1983; Fyfe, 1983) were obtained at 31.94 MHz using 2-ms, 50-kHz ^{1}H–^{13}C spin-lock contacts with high-power (65 kHz) proton dipolar decoupling. Samples were spun at the magic angle (54.7°) with respect to the static magnetic field in a double-bearing rotor system (Schaefer et al., 1987) at a rate of 3 kHz. The spectra of Figures 1–4 resulted from the accumulation of between 3000 and 40 000 transients each, with recycle delays ranging from 1 to 5 s. More specific information for each spectrum is provided in its figure caption.

Values of the proton rotating-frame relaxation time, $T_{1\rho}(H)$, are determined from the decay of carbon signal as a function of ^{1}H–^{13}C contact time (τ) in a CPMAS experiment (Schaefer and Stejskal, 1979). The experiment is performed in this way to take advantage of the spectral resolution of the ^{13}C NMR spectrum. The value $\langle T_{1\rho}(H)\rangle$ is calculated from a least-squares fit of log (carbon signal intensity) vs τ, using values of τ = 1.5–12 ms.

β-Cyclodextrin was purchased from Sigma Chemical Co.

Preparation of β-Cyclodextrin–Phenyl Ether Inclusion Complex 3. A solution of 11.35 g (0.01 mol) of β-cyclodextrin (2) in 400 mL of water was prepared by warming slightly. To this solution was added 3.21 g (0.01 mol) of 1 (Moedritzer and Rogers, 1990) as a solid, and the mixture was stirred overnight. A precipitate formed, which was filtered and washed twice with water. Extraction of the filtrate with three successive 100-mL portions of ether gave 0.23 g of recovered 1. The solid was dried under vacuum at 50 °C, cooled, suspended in ether, and filtered to remove uncomplexed 1. The ether wash was repeated twice more, and the product was dried under vacuum to give 9.39 g of 3 as a white solid. Anal. Calcd for $C_{42}H_{70}O_{35}\cdot C_{11}H_7Cl_1F_3N_3O_3$: C, 43.70; H, 5.33; N, 2.89; Cl, 2.43. Found: C, 42.09; H, 5.94; N, 2.10; Cl, 1.93. The analysis observed was consistent with $2_{1.33}1_{1.00}(H_2O)_{6.26}$.

Reprecipitation of β-Cyclodextrin. The blank sample was prepared by dissolving β-cyclodextrin (2) in warm water, cooling, filtering, and drying under vacuum at 50 °C.

RESULTS AND DISCUSSION

The inclusion complex 3 was prepared as described under Experimental Procedures and was characterized by elemental analysis and solution NMR. The solid was only soluble in dimethyl sulfoxide, and its solution NMR spectrum appeared as a superposition of the spectra of 1 and 2. Such a superposition of spectra would result if (1) 3 was the true inclusion complex which was not stable in solution, (2) 3 was the product of some other association of 1 and 2 which was not stable in solution, or (3) 3 was a physical mixture of 1 and 2. Solution NMR could not therefore prove that an inclusion complex had formed.

The X-ray powder diffractogram of the solid product was indeed different from that of either the starting cyclodextrin 2 or the starting ether 1. However, a third powder diffractogram of 2 that had been dissolved in water

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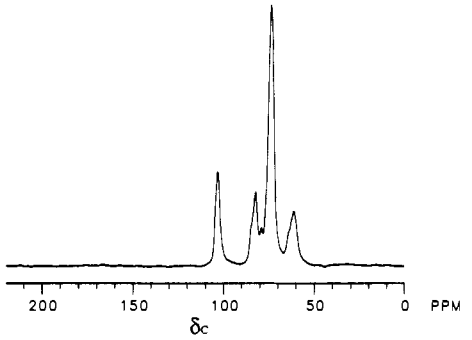

Figure 1. CPMAS ^{13}C NMR spectrum at 31.94 MHz of β-cyclodextrin (**2**), 394 mg, 3000 transients. This spectrum was collected using a 2-ms 50-kHz ^{13}C–^{1}H spin-lock contact with high-power proton decoupling ($\gamma B_2(H)/2\pi$ = 65 kHz), magic-angle spinning at 3.0 kHz, and a 1-s recycle delay between transients.

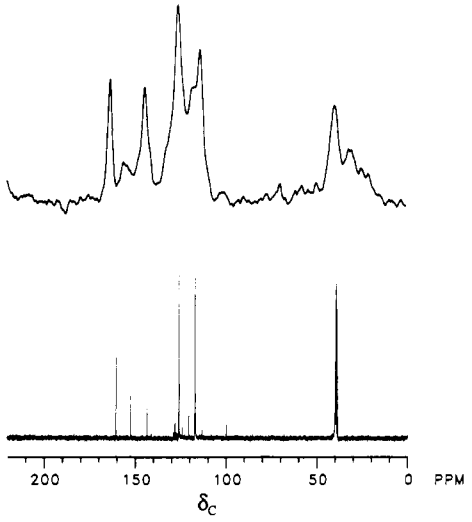

Figure 2. ^{13}C NMR spectra of heterocyclic phenyl ether **1**: (bottom) solution spectrum, at 75 MHz, in DMSO; (top) CPMAS spectrum, sample weight of 459 mg, 8000 transients. Experimental conditions are as listed in the caption to Figure 1, except that the recycle delay between transients was 5 s.

and then reprecipitated after cooling was completely different from either. X-ray powder diffraction failed to clarify the nature of the insoluble product. We endeavored to more fully characterize the complex **3** using cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR, a technique that we applied previously to analysis of a granular pesticide formulation (Garbow and Gaede, 1990).

Figure 1 shows the CPMAS ^{13}C NMR spectrum of β-cyclodextrin (**2**) as received. The four major resonances observed in this spectrum are assigned to the different sugar carbons in the glucose subunits of the cyclodextrin as C_6 (δ_C 61 ppm); C_2, C_3, C_5 (δ_C 74 ppm); C_4 (δ_C 82 ppm); and anomeric C_1 (δ_C 103 ppm). The sharp line at 79 ppm is of unknown origin, although its chemical shift and line width are suggestive of a carbon in a small sugar molecule.

Figure 2 shows the solution-state (bottom) and CPMAS (top) ^{13}C NMR spectra of the starting ether **1**. In a comparison of these spectra, the following characteristics of solid-state ^{13}C NMR spectra should be noted: (1) Because of the quadrupolar nature of the chlorine nucleus, resonances of carbons bonded to Cl are not observed, an effect that has been noted for halogenated polymers (Poliks and Schaefer, 1990). (2) Similarly, carbons that are bonded to nitrogen (another quadrupolar nucleus) show broadened, asymmetric lines (Olivieri et al., 1987; Hexem et al., 1981). (3) Finally, ^{13}C–^{19}F dipolar coupling makes the CF_3 carbon unobservable. All of the resonances

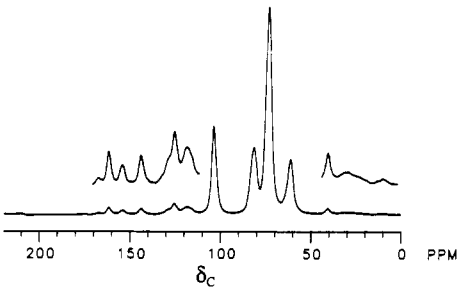

Figure 3. CPMAS ^{13}C NMR spectrum of the cyclodextrin inclusion complex, **3**, 381 mg, 40 000 transients. All experimental conditions are as listed in the caption to Figure 1.

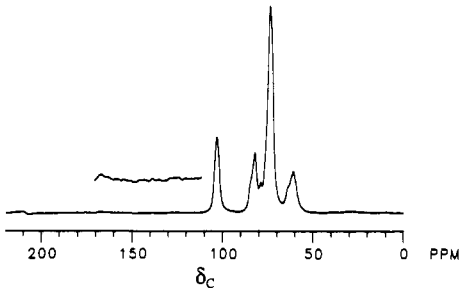

Figure 4. CPMAS ^{13}C NMR spectrum of a physical mixture (1:1 mol/mol) of β-cyclodextrin (**2**) and heterocyclic phenyl ether, **1**, 424 mg, 20 000 transients. All experimental conditions are as listed in the caption to Figure 1.

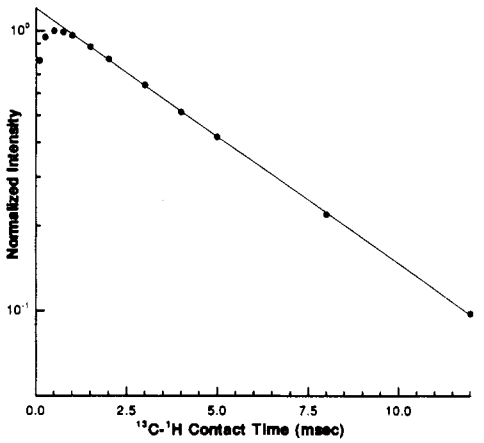

Figure 5. Semilog plot of ^{13}C NMR signal intensity vs CP contact time, τ, for the 74 ppm resonance in β-cyclodextrin (**2**). The straight line is a least-squares fit of the signal decay over the range τ = 1.5–12 ms, whose slopes yield the value of $\langle T_{1\rho}(H)\rangle$.

observed in the solid-state ^{13}C NMR spectrum correspond well with the solution NMR spectrum of **1**. The relatively poor signal-to-noise ratio of the solid-state spectrum results from the long T_1 relaxation times of the protons in this sample.

The spectrum of the inclusion complex **3** (Figure 3) shows all of the major resonances of **1** and **2**. The signals attributed to **2** are somewhat broader than in the spectrum of **2** alone, and some of the resonances attributed to **1** have shifted in position and relative intensity, suggesting that **1** and **2** are interacting in some way. The mere observation of resonances due to **1** in the inclusion complex provides additional evidence for complex formation. This is illustrated by Figure 4, the CPMAS ^{13}C NMR spectrum of a 1:1 (mole/mole) physical mixture of **1** and **2**. The absence of resonances due to **1** in the spectrum of the physical mixture is due to the long T_1 relaxation times of the protons in **1**. Interaction between **1** and **2** in the inclusion complex shortens this proton T_1, and signals from the carbons of **1** are observed.

Table I. $T_{1\rho}$(H) Values for the Various β-Cyclodextrin Samples

	⟨$T_{1\rho}$(H)⟩, ms							
	β-cyclodextrin (2) resonances				phenyl ether (1) resonances			
sample	61 ppm	74 ppm	82 ppm	103 ppm	125 ppm	144 ppm	154 ppm	162 ppm
β-cyclodextrin (as received)	4.9	4.8	4.5	4.9				
β-cyclodextrin (reprecipitated)	6.3	5.9	6.2	6.1				
β-cyclodextrin/1 inclusion complex	6.1	5.8	6.0	5.8	7.1	6.2	8.7	6.6
β-cyclodextrin/1 physical mixture	4.4	4.6	4.7	4.6				

To investigate the interaction of 1 and 2 in greater detail, we measured the proton rotating-frame relaxation time, $T_{1\rho}$(H), for several different samples. As described under Experimental Procedures, $T_{1\rho}$(H) is measured by monitoring the decay of ^{13}C signal as a function of the ^{13}C–^{1}H contact time (τ) in a cross-polarization experiment. $T_{1\rho}$(H) is sensitive to kilohertz regime motions and has been used extensively to characterize motions in polymers (Stejskal et al., 1979). $T_{1\rho}$(H) can also provide information about domain formation and phase separation in solids. In solids, efficient proton–proton communication among protons that are proximate in space, a process referred to as spin diffusion (Komoroski, 1986), causes them to behave as a single spin reservoir, characterized by a single, averaged ⟨$T_{1\rho}$(H)⟩. This averaging has been used to assess the goodness of mixing in polymer blends (Stejskal et al., 1981) and, recently, to characterize the distribution of protein and starch components in wheat flours (Garbow and Schaefer, 1991). By contrast, the measurement of two or more different ⟨$T_{1\rho}$(H)⟩ values in a solid sample indicates the presence of distinct proton spin reservoirs which are separated from one another in space.

Figure 5 shows a plot of carbon signal intensity vs CP contact time, τ, for the 74 ppm resonance of β-cyclodextrin, as received. The results of our $T_{1\rho}$(H) experiments are summarized in Table I. The first two rows of this table list values of ⟨$T_{1\rho}$(H)⟩ for two different samples of β-cyclodextrin. As expected, we measure the same value of ⟨$T_{1\rho}$(H)⟩ no matter which carbon resonance we monitor in these samples. The minor difference observed between ⟨$T_{1\rho}$(H)⟩ for the "as received" and recrystallized samples is probably due to minor differences in the packing or water content of these solids. Line 3 of Table I presents $T_{1\rho}$(H) results for the inclusion complex 3. ⟨$T_{1\rho}$(H)⟩ values for the cyclodextrin resonances are the same as in the pure compound and are similar to those measured for the signals of 1. This is in marked contrast to the relaxation behavior of 1 in its pure solid form. Although the long T_1 relaxation time prevented a measurement of ⟨$T_{1\rho}$(H)⟩ for the sample of 1, we did measure carbon signal intensities at two different ^{13}C–^{1}H contact times: 2 and 12 ms. The results (data not shown) indicate that signal intensities for 1 are actually marginally greater at 12-ms contact than at 2 ms. This is in marked contrast to the inclusion complex, where the signal from 1 is approximately 70% decayed after 12 ms.

Another observation from the variable contact time experiment is that the carbons of 1 cross-polarize slowly. This is indicative of attenuated ^{13}C–^{1}H dipolar couplings and is unexpected for a proton-rich organic solid. It is, however, exactly what would be expected for an inclusion complex—1 inside the β-cyclodextrin cage is free to undergo considerable motion, which reduces the ^{13}C–^{1}H dipolar couplings. This motion of 1 within the β-cyclodextrin cage could also account for the small difference observed in ⟨$T_{1\rho}$(H)⟩ values as measured through the carbons of 1 and 2 (Table I, line 3).

With the characterization of the complex 3 on firm ground, we turned to its biological activity. Unfortunately, no significant difference in biological activity of the complex 3 vs the uncomplexed phenyl ether 1 was observed other than a dilution of unit activity which was proportional to the dilution of active ingredient by the cyclodextrin. The biological activity was measured using a multispecies whole plant assay, both pre- and postemergent. A series of rates were tested to determine a 50% effective dose for the test compounds.

CONCLUSIONS

In total, the ^{13}C NMR relaxation data suggest an intimate mixing of 1 and β-cyclodextrin (2) on a molecular scale, consistent with the formation of an inclusion complex. The broadening of the signals of 1 in this sample is also consistent with complex formation. The data rule out this sample being a physical mixture of 1 and 2.

CPMAS ^{13}C NMR spectroscopy was the only analytical technique investigated that gave an unequivocal characterization of the complex. Results obtained with X-ray powder diffraction could not distinguish between formation of an inclusion complex and precipitation of an altered crystalline form of β-cyclodextrin. On the basis of these results, we expect that solid-state ^{13}C NMR spectroscopy will find increasing use in the characterization of inclusion complexes.

Supplementary Material Available: X-ray diffractograms of β-cyclodextrin (2), as received and reprecipitated, and of complex 3 (3 pages). Ordering information is given on any current masthead page.

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