

PHOTOSTABILIZATION OF A NITROMETHYLENE HETEROCYCLE INSECTICIDE ON THE SURFACE OF MONTMORILLONITE

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Abstract—The photochemical stability of the insecticidal compound tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH) adsorbed on montmorillonite (Mont), in the presence or in the absence of a second organic chromophore was studied. Two different organic dyes were investigated as possible stabilizers of NMH: the divalent cation methyl green (MG) and the monovalent cation thioflavin T (TFT). Samples of free NMH and of the adsorption complexes Mont-NMH, Mont-MG-NMH, and Mont-TFT-NMH were exposed to direct sunlight, and the residual insecticidal activity was estimated. Some photostabilization of the pesticide adsorbed to the clay was observed. The highest degree of photoprotection was achieved in samples containing 0.5 mmole of TFT and 0.2 mmole of NMH/g clay. Increasing the load of TFT to 0.8 mmole/g clay resulted in a complete loss of photostabilization. The interactions of the organic molecules at the clay surface were studied by UV-VIS absorption and Fourier-transform infrared spectroscopy. For the Mont-NMH and Mont-MG-NMH complexes, the observed photostabilization is probably due to clay-NMH interactions. In the Mont-TFT-NMH complex specific interactions between the cationic dye and the pesticide molecules probably occurred as well.

Key Words—Adsorption, Clay-organic complex, Infrared spectroscopy, Montmorillonite, Nitromethylene heterocycle, Pesticide, Photostabilization.

INTRODUCTION

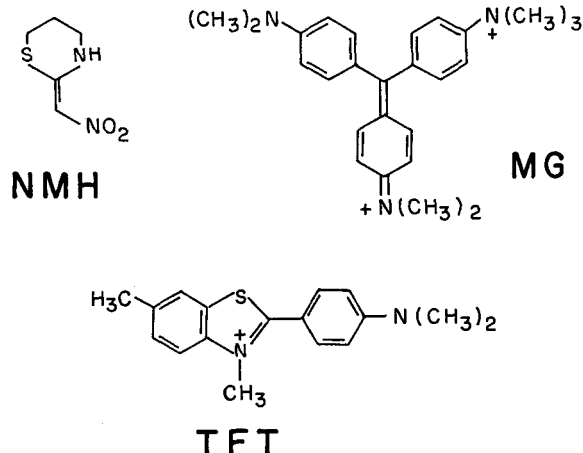
Studies of the nature of clay-organic interactions (Mortland, 1970; Theng, 1974) have established the basis for investigations of energy transfer processes between molecules adsorbed at clay surfaces (Krenske *et al.*, 1980; DellaGuardia and Thomas, 1983a, 1983b, 1984; Schoonheydt *et al.*, 1984; Nakamura and Thomas, 1985, 1986; Avnir *et al.*, 1986). Margulies *et al.* (1985) suggested the possibility of utilizing such interactions for stabilizing photolabile agrochemicals.

Sensitivity to sunlight is a principal factor that inhibits the effective application of many pesticides. Despite the high insecticidal activity of some nitromethylene heterocyclic compounds, they are of limited practical use due to their extreme photolability. Of these materials, tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH) displays the highest biological activity (Soloway *et al.*, 1978). Photochemical studies have established the identity of several photoproducts that are modifications mediated by the nitrovinyl group, which is essential for insecticidal activity (Kleier *et al.*, 1985). In the latter work the conclusion has been that photostabilization may not be achieved merely by structural alterations of the pesticide molecules, but requires instead the use of external additives. The use of UV-absorbing materials as additives in commercial for-

mulations has been the conventional approach to photostabilizing pesticides. The major drawback of such additives is related to the negative ecological impact introduced by the large amounts required for significant photoprotection.

The new method of photostabilizing pesticides suggested by Margulies *et al.* (1985) involves adsorption on the surface of clays of the biologically active molecule and another organic chromophore. The chromophore is selected to deactivate the photoexcited pesticide molecules by efficient energy transfer processes before photodegradation starts. This method has been recently used to photostabilize the insecticidal pyrethroid bioresmethrin using two different organic cations adsorbed on montmorillonite (Margulies *et al.*, 1987).

In the present investigation the intermolecular interactions on the surface of montmorillonite of photolabile NMH and two different organic dyes, considered as possible stabilizers, have been studied. The dyes were the divalent cation methyl green (MG), shown previously (Margulies *et al.*, 1987) to be effective in photostabilizing bioresmethrin, and the monovalent cation thioflavin T (TFT). The improvement of the insecticidal life time of NMH exposed to sunlight by these interactions is also reported. The formulae of NMH, MG, and TFT are shown below.



MATERIALS AND METHODS

Materials

The clay used was Na-montmorillonite SWy-1 obtained from the Source Clays Repository of The Clay Minerals Society. NMH (Shell, SD 35651) was supplied by L. O. Ruzo of the University of California, MG was obtained from Fluka AG, and TFT was obtained from Aldrich Chemical Company. These chemicals were used without further purification.

Preparation of clay-organic complexes

The clay-dye complexes montmorillonite-MG (Mont-MG) and montmorillonite-TFT (Mont-TFT) were prepared by dropwise addition of aliquots of 10^{-3} M aqueous solutions of the cations to a 0.5% aqueous suspension of the clay under continuous stirring. After 30-min centrifugation at 16,000 g, the precipitate was washed three times with distilled water, freeze-dried, and ground to $<50 \mu\text{m}$. Attempts to measure the concentration of the cationic dye in the supernatant by UV-VIS spectroscopy failed because in all the cases no absorption could be detected. This indicates that all the added dye was adsorbed by the clay. The infrared spectra of the clay-dye complexes showed a weakening of the absorption bands of water at about 1640 cm^{-1} and 3400 cm^{-1} with increasing loads of dye. This dehydration effect, together with the X-ray powder diffraction data shown in Table 1 for Mont-TFT and that previously reported for Mont-MG (Margulies and Rozen, 1986) prove that the dye molecules are intercalated between the montmorillonite layers.

To prepare the Mont-NMH, Mont-MG-NMH, and Mont-TFT-NMH complexes, the appropriate volume of a 10^{-2} M solution of NMH in hexane was added to the powdered clay, Mont-MG, and Mont-TFT, respectively, and the solvent was evaporated under reduced pressure.

Table 1. Basal spacing of montmorillonite-thioflavin T complexes.

Sample ¹	c-spacing (Å)	
	Air-dried	Oven-dried ²
Mont	15.1	13.5
Mont-TFT _{0.5}	15.5	14.8
Mont-TFT _{0.8}	15.5	15.1

¹ Mont = montmorillonite; TFT = thioflavin T; subscripts of TFT refer to its concentration in the complex (in mmole/g clay).

² Heated 24 hr at 105°C.

Irradiation

For irradiation treatment, 10 mg of powdered clay-organic complex was homogeneously spread on the bottom of Petri dishes (5 cm in diameter). To examine the free insecticide, NMH dissolved in hexane was applied to the Petri dish and the solvent subsequently evaporated. The insecticide-dye mixtures were prepared by evaporating a methanol solution of NMH with either MG or TFT on a Petri dish. The samples were exposed to direct sunlight for various lengths of time; dishes covered with aluminum foil served as controls. Irradiation experiments were carried out on clear sunny days in the fall of 1985 for methyl green complexes and in the summer of 1986 for thioflavin T complexes. Samples of Mont-NMH were included in both experiments. On each day of the experiment new controls of free NMH were exposed to sunlight to verify photoinactivation of the pesticide. Days on which the inactivation of the controls was incomplete were not included in the exposure time.

Bioassay

Bioassay experiments were carried out using adults of the flour beetle *Tribolium castaneum* (SCbb strain) (Margulies *et al.*, 1987). All samples containing NMH, either free or adsorbed on clay, were biologically active before exposure to sunlight, whereas all other components (clay, MG, or TFT) were inactive. The “% mortality” values presented are mean values calculated from three replicates.

Spectroscopic and X-ray powder diffraction measurements

UV-VIS absorption spectra of the aqueous solutions of NMH, MG, TFT, and a Nujol mull of Mont-NMH were measured in a Uvikon 810 spectrophotometer. Fourier-transform infrared (FTIR) spectra of the pure compounds and of the adsorption complexes were measured in KBr pellets using a Nicolet MX-S spectrophotometer interfaced to an Elite Star 16-bit PC and a Goerz SE 284 digital plotter. X-ray powder diffraction measurements were carried out in a Philips 1030 diffractometer using the $\text{CoK}\alpha$ line.

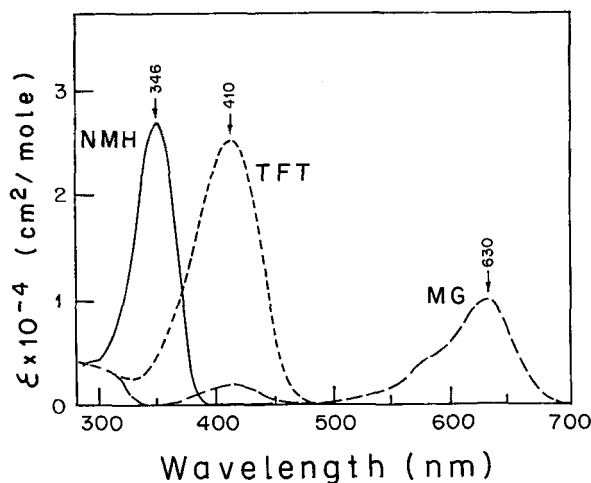


Figure 1. Electronic absorption spectra of aqueous solutions of tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH), thioflavin T (TFT), and methyl green (MG).

RESULTS AND DISCUSSION

The long wavelength band in the UV absorption spectrum of NMH dissolved in water has its maximum at 346 nm (Figure 1). This band was assigned to an electronic transition involving the transfer of π electron density from the enamine portion of the molecule to the nitro group (Kleier *et al.*, 1985). The absorption spectra of the two dyes, MG and TFT, in aqueous solutions are also shown in Figure 1. TFT has a strong absorption at about 410 nm, and MG exhibits its strongest absorption at much longer wavelengths ($\lambda_{\max} = 630$ nm). These differences were expected to affect the efficiency of possible energy transfer processes between pesticide and organic chromophore.

Results of typical bioassays aimed at evaluating MG and TFT as potential photoprotectors of NMH are shown in Figures 2 and 3, respectively. Despite some variations in % mortality, which was related to the method of bioassay used, the trends are evident. Free NMH lost its insecticidal activity during the first day of irradiation. Mixtures of NMH and MG or TFT were also inactive after one day of exposure to sunlight (not shown). In both experiments, some photostabilization was observed in samples in which the pesticide was adsorbed on montmorillonite at a load of 0.2 mmole NMH/g clay (Mont-NMH_{0.2}). The photostabilization was slightly higher in samples containing MG coadsorbed with the pesticide on the surface of the clay at 0.2 mmole each/g clay (Mont-MG_{0.2}-NMH_{0.2}, Figure 2).

Greater photostability was achieved by using TFT as the protecting chromophore. Montmorillonite containing 0.5 mmole of TFT and 0.2 mmole of NMH adsorbed/g clay (Mont-TFT_{0.5}-NMH_{0.2}, Figure 3) re-

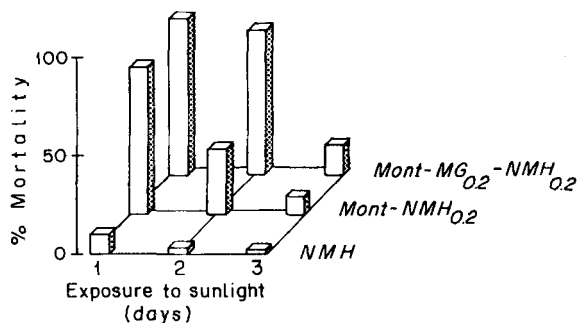


Figure 2. Evaluation of the photostabilization of tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH) by methyl green (MG) and montmorillonite (Mont), using the *Tribolium castaneum* bioassay. Subscripts of NMH and MG refer to their concentrations in the complexes (in mmole/g clay).

tained high insecticidal activity after four days of exposure to sunlight. The photostabilizing effect disappeared when the insecticide was adsorbed to a clay containing 0.8 mmole TFT/g, i.e., whose cation-exchange capacity had been saturated by the organic cation (Mont-TFT_{0.8}-NMH_{0.2}, Figure 3).

These results suggest that, besides a possible light-attenuation effect by the clay particles, two main factors contributed to the quenching of the photochemical degradation of NMH: (1) interaction between the pesticide molecules and the clay; and (2) interaction between the pesticide molecules and the TFT molecules at the surface of the mineral. A possible explanation for changes in photochemical reactivity of pesticides adsorbed on clay surfaces is based on the shift of their absorption bands to <290 nm (short wavelength limit of the solar radiation) (Plimmer, 1972). This explanation is considered unlikely on the basis of the absorption spectrum of Mont-NMH_{0.2} measured in Nujol mull (Figure 4). As in the spectrum of free NMH in solution (Figure 1a), the strong absorption band of NMH is at about 360 nm (on a scattering background of montmorillonite) and has a minimum absorption at 300 nm. The absorption band of NMH in the adsorbed state is red-

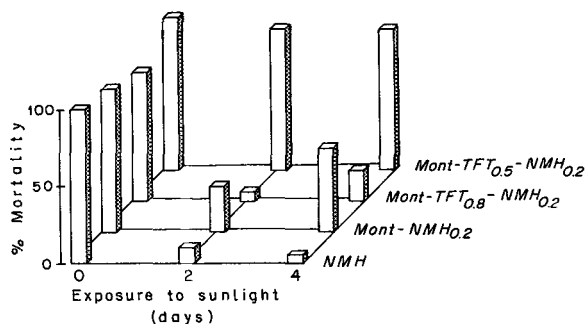


Figure 3. Evaluation of the photostabilization of tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH) by thioflavin T (TFT) and montmorillonite (Mont), using the *Tribolium castaneum* bioassay. Subscripts of NMH and TFT refer to their concentrations in the complexes (in mmole/g clay).

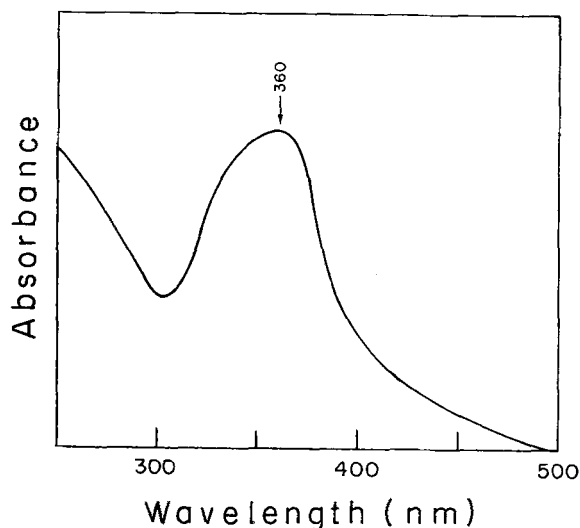


Figure 4. Electronic absorption spectra of tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH) adsorbed on montmorillonite (0.2 mmole/g clay) measured in Nujol mull between two quartz plates.

solution. This red shift indicates, relative to the situation in an aqueous solution, that the interactions of NMH molecules with the clay were stronger in the excited state than in the ground state. Such clay-NMH interactions were probably involved in the observed photostabilization of the pesticide. Loading the clay surface with large amounts of TFT (Mont-TFT_{0.8}-NMH_{0.2}) presumably impaired clay-NMH interactions and, therefore, prevented photostabilization.

Two possible mechanisms can be considered regarding the photoprotection by the organic dye: (1) competitive absorption of UV light; and (2) specific intermolecular interactions between the excited pesticide molecules and the cationic dye. The low absorbance of TFT at about 350 nm (Figure 1) and the fact that photoprotection was lost in samples containing larger amounts of this dye adsorbed (Mont-TFT_{0.8}-NMH_{0.2}, Figure 3) strongly suggest that photostabilization was mainly due to the second mechanism.

Fourier-transform infrared (FTIR) spectra in the 1200–1800-cm⁻¹ region are presented in Figure 5 for NMH adsorbed on montmorillonite (Mont-NMH_{0.2}, curve b), for Mont-MG_{0.2}-NMH_{0.2} (curve c), and for Mont-TFT_{0.5}-NMH_{0.2} (curve d). In addition, the spectrum of the clay without any organic compound adsorbed is also shown (curve a). Although some characteristic absorption bands of NMH can be seen in curve b, they are obscured by absorptions of the dye molecules in spectra c and d.

To facilitate comparison of the vibrational spectra, the differential FTIR spectra of adsorbed NMH are presented in Figure 6 together with the spectrum of the "free" compound (Figure 6a). Difference spectra were obtained by subtracting from the spectra of complexes

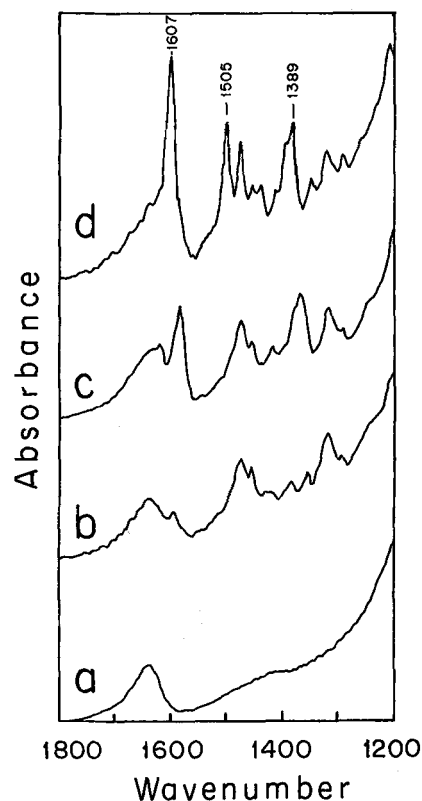


Figure 5. Fourier-transform infrared absorption spectra of (a) montmorillonite; (b) tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH) adsorbed on montmorillonite (0.2 mmole/g clay); (c) NMH and methyl green co-adsorbed on montmorillonite (0.2 mmole each/g clay); (d) NMH and thioflavin T co-adsorbed on montmorillonite (0.2 and 0.5 mmole/g clay, respectively).

containing NMH the spectra of the corresponding supports without adsorbed pesticide.

Pronounced differences were observed between the shifted relative to the corresponding band in aqueous FTIR spectra of free and of adsorbed NMH (Figures 6a and 6b). Worth noting is the absence of the two strong bands at 1234 cm⁻¹ and at 1287 cm⁻¹, which were assigned to enamine C–N stretching vibrations. Drastic changes were also noted in the position and intensity of peaks between 1350 and 1500 cm⁻¹, corresponding mainly to C–H bending vibrations (Nakanish and Soloman, 1977; Pouchert, 1981). The symmetric and antisymmetric stretching of the nitro group at 1317 and 1594 cm⁻¹, respectively (Nakanish and Soloman, 1977; Pouchert, 1981), were only slightly affected by adsorption. Thus, the main interaction between the clay surface and the pesticide appears to have taken place through the cyclic enamine part of the NMH molecule, rather than through the nitro group.

The spectrum of NMH adsorbed on Mont-MG_{0.2} (Figure 6c) is almost identical to that of NMH adsorbed on the clay alone (Figure 6b), suggesting that the interactions of the pesticide molecules did not depend

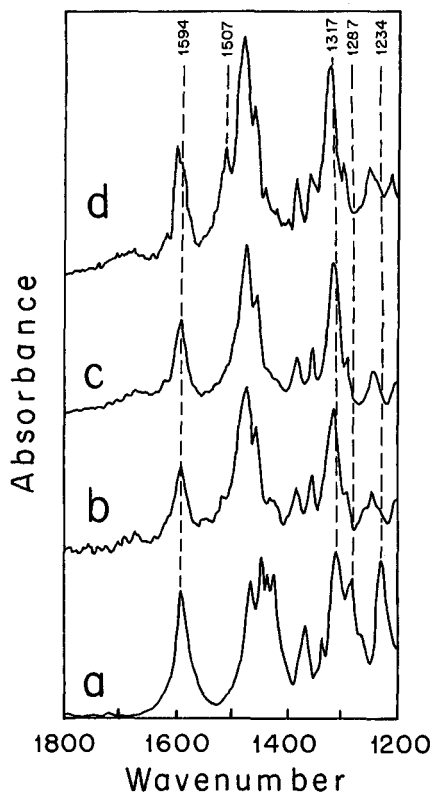


Figure 6. Fourier-transform infrared absorption spectra of tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (NMH): (a) free; (b–d) adsorbed on montmorillonite (Mont)-organic complexes. Curves b–d were obtained by subtracting the following spectra: (b) (Mont-NMH_{0.2})-(Mont); (c) (Mont-MG_{0.2}-NMH_{0.2})-(Mont-MG_{0.2}); (d) (Mont-TFT_{0.5}-NMH_{0.2})-Mont-TFT_{0.5}). Subscripts of NMH, MG, and TFT refer to their concentrations in the complexes (in mmole/g clay).

on the presence or absence of adsorbed methyl green. For NMH adsorbed on Mont-TFT_{0.5}, however, an additional peak at 1507 cm⁻¹ and a broadening to higher frequencies of the 1594 cm⁻¹ band were observed (Figure 6d). These two frequencies are close to the strong absorptions of TFT at 1505 cm⁻¹ and 1607 cm⁻¹ (aromatic ring vibrations) (Figure 5d). The possibility that an incorrect normalization factor was used in the spectrum subtraction is unlikely because the third strong peak of TFT in this spectral region (1389 cm⁻¹, Figure 5d) disappeared in the difference spectra. Therefore, the additional features in the spectrum originated from changes in the absorption spectrum of either NMH or TFT due to their coadsorption at the surface of the clay, indicating specific interactions between the two organic molecules. Similar interactions were found for a complex of montmorillonite, MG, and the synthetic pyrethroid bioresmethrin (Margulies *et al.*, 1987).

The results presented suggest that interactions between NMH and montmorillonite and between adsorbed NMH and TFT at the surface of the clay played

an important role in the observed photostabilization of the pesticide. Excessive coverage of the clay surface by the protecting dye perturbed the balance of these interactions and resulted in loss of photostability.

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