Phygon. Fate of 2,3-Dichloro-1,4-naphthoquinone in Crop Extracts

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The major steps involved in updating analytical procedures for determining fungicide residues, extraction, storage, purification, detection, and confirmation of identity are discussed as they relate to Phygon. Conversion products are identified and a synthesis route is given for obtaining 2-chloro-3-phenyl-1,4-naphthoquinone in good quantity.

Phygon, 2,3-dichloro-1,4-naphthoquinone, commonly known as dichlone, is used extensively as an organic fungicide on a variety of fruits and vegetables. The compound, a bright yellow crystalline material (mol. wt. 227, m.p. 193° C.), is commercially prepared by chlorination of 1,4-naphthoquinone in glacial acetic acid (Lane, 1964).

Present analytical procedures for the determination of Phygon residues are based on the colorimetric methods developed by Burchfield and McNew (1948) and modified by Lane (1958). This paper describes a gas chromatographic procedure for monitoring Phygon residues, including an examination into the effect of storage and light on Phygon in solution, and the isolation, structure elucidation, and synthesis of the corresponding photoconversion products.

MATERIALS AND EQUIPMENT

Chemicals. Phygon was obtained from the U. S. Rubber Co., Naugatuck Chemical Division, Naugatuck, Conn.

Hexane, acetone, chloroform, and benzene were doubled distilled reagent grade quality solvents.

Other reagents included technical grade 1,4-naphthoquinone, anhydrous sodium acetate, reagent grade aniline, glacial acetic acid, reagent grade hydrochloric acid (38%), high purity chlorine gas, 95% ethanol, cupric chloride, and sodium nitrite.

Gas Chromatographs. Several gas chromatographs were utilized during this investigation. The Aerograph HY-FI Model 600B (Varian Aerograph, Walnut Creek, Calif.) was equipped with an electron-capture detector and a 5-foot × ⅛-inch o.d. borosilicate glass column packed with 5% QF-1 (w./w.) on 70- to 80-mesh acid-washed Chromosorb G treated with DMCS. The F&M Model 810 gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) was equipped with a 10-to-1 effluent splitter, differential flame ionization detector, and dual 6-foot × ⅛-inch o.d. borosilicate glass columns packed with 5% QF-1 (w./w.) on 70- to 80-mesh acid-washed Chromosorb G treated with DMCS. The Dohrmann Model G-100 (Dohrmann Instrument Co., Mountain View, Calif.) was equipped with a Model C-200 microcoulometer using a halogen cell and a 5-foot × ¼-inch o.d. borosilicate glass column packed with 10% SE-30 (w./w.) on 60- to 80-mesh acid-washed Chromosorb W.

Spectrophotometers. Infrared spectra were determined from potassium bromide disks, utilizing a Perkin-Elmer Model 337 spectrophotometer. Visible spectra were measured in benzene solutions in a Beckman DK-2A recording spectrophotometer.

Spectrometers. Mass spectra were acquired through the use of a Varian Model M-66 cyclodial double-focusing mass spectrometer. The samples were introduced into the instrument with a direct sample introduction probe. Nuclear magnetic reasonance spectra were determined with a Jeolco Model JNM C-60 instrument, and a Varian Model T-60 instrument coupled to a Varian Model C-1024 time averaging computer. Samples were prepared as deuterochloroform solutions with tetramethylsilane as an internal standard.

EXPERIMENTAL

Extracts from a variety of fruits were examined for Phygon residues by electron-capture gas chromatography. The field-treated samples were macerated and extracted in a routine manner with benzene (Kilgore and White, 1967). The benzene extracts were dried over anhydrous sodium sulfate and stored until analyzed. A 5-μl. aliquot of each extract was injected into the gas chromatographic column to determine the need for a cleanup procedure. When required, a Florisil cleanup procedure was useful in purifying benzene extracts of cherries, peaches, and apricots (Miller, 1965).

Fortifications of check samples for recovery studies were made prior to maceration and solvent extraction. The recovery rate of fortified samples appeared to fall as the fortification level was lowered and as the length of time between extraction and analysis increased. The latter observation was reported earlier by Lane (1958). Figure 1 represents an approximation of both observations.

The standard curve of a freshly prepared benzene solution of Phygon was linear over a range equivalent to 0.3 to 5.0 ng. of Phygon. The per cent deviation for a replicate series of 10 injections, each being equivalent to 1.5 ng. (1.5-μl. aliquots) of phygon was ±2.5. The gas chromatographic characteristics and electron-capture response of this series of Phygon determinations is illustrated in Figure 2.

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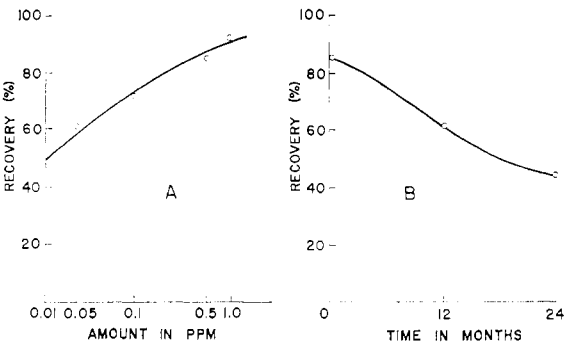


Figure 1. Recovery of Phygon from crop extracts

A. Curve represents recoveries of Phygon obtained from fortified crops which were extracted and analyzed during the same day by electron-capture gas chromatography

B. Degradation curve of 0.5 p.p.m. of Phygon in crop extracts. Each point represents average of three determinations of three different fruits. Extracts were stored in sealed bottles under ordinary laboratory conditions of light and temperature

In contrast, Figure 3 represents the same standard solution subjected to electron-capture gas chromatography after 12 months under ordinary laboratory conditions of light and temperature. The concentration of Phygon decreased concomitantly with increases in the concentrations of degradation and conversion products. The same effect was produced by exposing the freshly prepared benzene solution of Phygon to 6 hours of natural sunlight. A reference solution of Phygon (1 mg. per ml.) stored in the dark at room temperature for a 12-month period showed very little degradation.

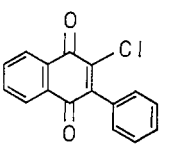
Isolation and Identification of the Photoproducts. Aliquots (10-μl.) of the concentrated, irradiated solution of Phygon were injected into the F&M gas chromatograph, and the effluents corresponding to the numbered peaks in Figure 3 were collected separately. Capillary tubing positioned at the exit port of the heated thermal conductivity detector served as a convenient trap. Re-injection of each separately collected product showed only a single response curve on the gas chromatograph.

Infrared spectra were determined for each of the purified compounds. Compound 1 was easily identified from its infrared spectra as the anhydride form of phthalic acid. Verification was made by injecting a phthalic acid solution into the gas chromatograph, collecting the corresponding effluent and comparing its infrared spectra to that of Compound 1. Similarly, Compound 2 was confirmed to be Phygon from its retention time and infrared spectra.

Positive identification of Compound 3, the most abundant photoconversion product, was possible after subjecting the compound to a number of different spectral measurements. The infrared spectra of Phygon and its photoproduct are presented in Figure 4.

A comparison of the photoproduct with that of Phygon shows a shift in the carbonyl absorption of Phygon (1690 cm.$^{-1}$) to a lower frequency (1675 and 1665 cm.$^{-1}$). This fact, coupled with the appearance of the carbonyl doublet, strongly suggested the availability of two nonequivalent carbonyls. The photoproduct includes additional absorption bands (695 and 758 cm.$^{-1}$) which might be assigned to a monosubstituted benzene ring system as described by Nakanishi (1962).

The ascertainment of chlorine in the molecule was obtained by injecting a small benzene aliquot of the pure compound into the Dohrmann gas chromatograph. The halogen cell responded to a single compound which was unquestionably chlorinated. The actual number of chlorine atoms in the molecule was then determined from the mass spectrum of the photoproduct (Figure 5). In general, the number of chlorine atoms in a molecule can be determined by the number and the intensities of alternate peaks beyond the parent peak. A compound that contains one chlorine atom will have a P + 2 peak approximating one third the intensity of the parent peak because of the presence of molecular ions containing the ^{37}Cl isotope (Silverstein and Bassler, 1967). Furthermore, the most abundant fragment in the spectrum, at m/e 233.16, can be attributed to the loss of one chlorine atom. On the basis of collected physical evidence, the major photoproduct of phygon was assigned the following proposed structure and nomenclature.



2-chloro-3-phenyl-1,4-naphthoquinone

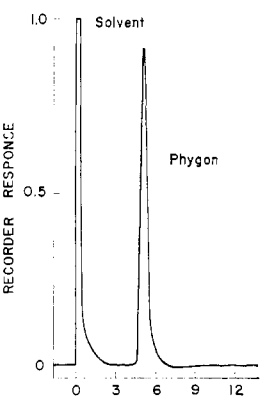


Figure 2. Gas chromatogram of Phygon

Curve represents 1.5 ng. of Phygon

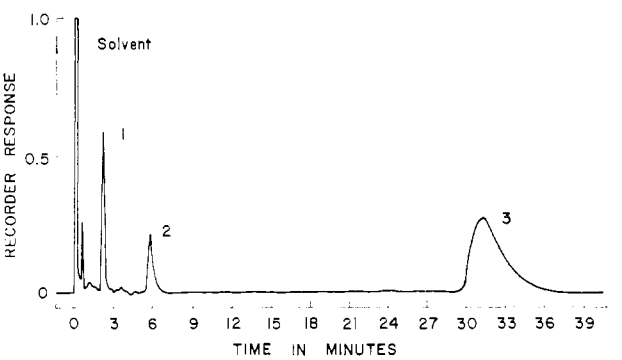


Figure 3. Gas chromatogram of irradiated Phygon

Curve represents 1.5 ng. of Phygon and Phygon conversion products

The calculated mass of the proposed structure (268.03) compared favorably with the mass of the photoproduct (268.04 ± 0.01). Also, the fragmentation pattern of the photoproduct proved consistent with the estimated fragmentation pattern of the proposed structure (Bowie *et al.*, 1965; Di Mari *et al.*, 1966). Further supporting evidence concerning the proposed structure of the photoproduct was obtained from the NMR spectrum of the compound (Figure 6A). The spectrum represents the summation of 302 scans of a 2-mg. sample with the T-60 spectrometer and C-1024 time averaging computer. The integral of the accumulated 302 scans, although not completely resolved, shows roughly a 2-2-5 relationship for the 3 bands of signals. The spectrum readout indicated positions relative to TMS at 0.

Synthesis of 2-Chloro-3-phenyl-1,4-naphthoquinone. A scheme for the preparation of 2-chloro-3-phenyl-1,4-naphthoquinone is shown in Figure 7. The synthesis of this compound, utilizing Meerwein's free radical arylation reaction, proved simple and usually resulted in reasonably good yields. All intermediates formed during the over-all synthesis were purified before being used in a subsequent step. The final product was extracted from the reaction mixture with chloroform and recrystallized several times from ethanol. The purified compound was finally isolated in the form of fine golden yellow crystals. Gas chromatography revealed a single component having the same retention time as the corresponding Phygon photoproduct. Final characterization was made through infrared spectra, mass spectra, and NMR spectra. Figure 6B illustrates the NMR spectrum of the synthesized compound. The spectrum represents a single scan of a 5-mg. sample with the JNM C-60 spectrometer.

The reaction between 2-chloro-3-phenyl-1,4-naphthoquinone and anhydrous dimethylamine produces a reddish-orange color which interferes with the absorption maxima of the orange product formed by the reaction of Phygon and dimethylamine. A comparison of the absorption spectra of both products is given in Figure 8.

DISCUSSION

Residue investigations should be specifically designed to encompass the total residue picture—i.e., "toxic degradation, metabolic, or other conversion products should

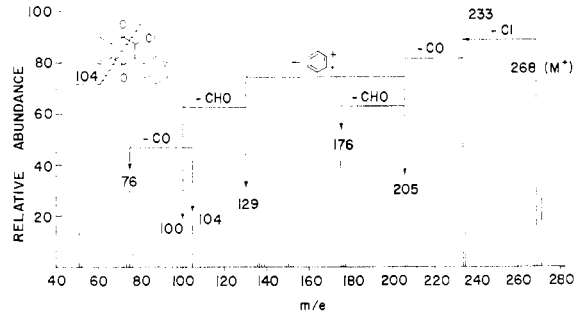


Figure 5. Mass spectrum of Phygon photoproduct

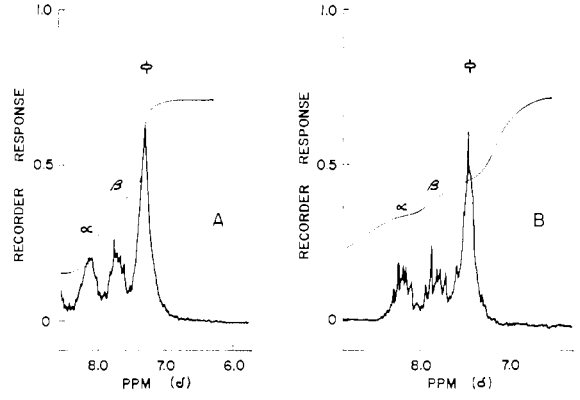


Figure 6. NMR spectra of (A) Phygon photoproduct and (B) 2-chloro-3-phenyl-1,4-naphthoquinone

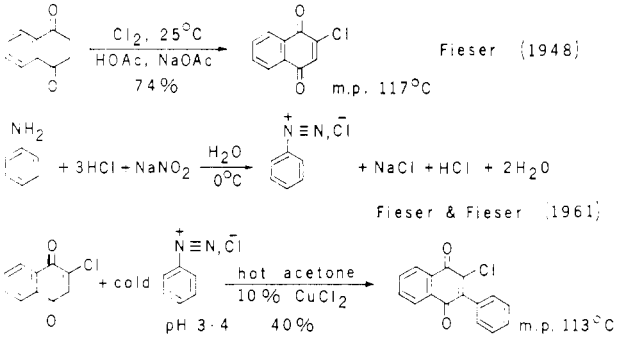


Figure 7. Synthesis of 2-chloro-3-phenyl-1,4-naphthoquinone

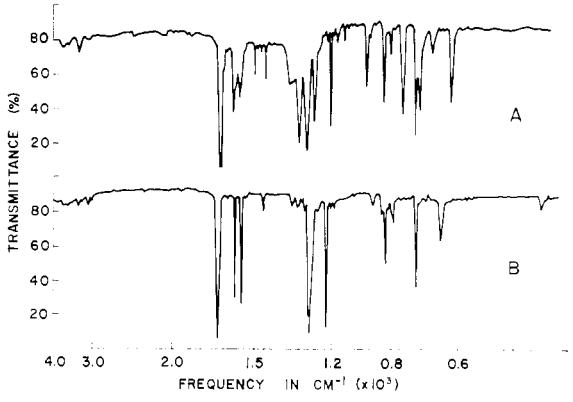


Figure 4. IR spectra of (A) Phygon photoproduct and (B) Phygon

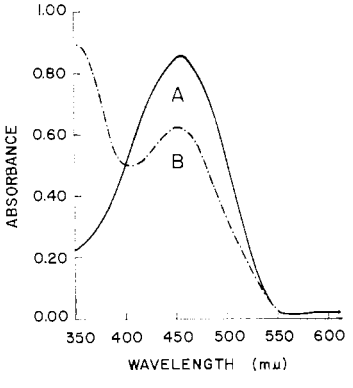


Figure 8. Absorption spectra of (A) orange color produced by reaction of Phygon with dimethylamine and (B) reddish-orange color produced by reaction of Phygon photoproduct with dimethylamine

be included in the residue determination" (FDA, 1968). Furthermore, the method should be sufficiently specific to identify and measure residues of other pesticides which reasonably could be expected to be present on the same commodities. The residue analytical procedure used for this study was designed from a gas chromatographic procedure reported by Kilgore and White (1966), involving the separation and identification of 18 different fungicides, including Phygon, from a single injection.

Obviously, the samples should be analyzed as quickly as possible after extraction, but even with this precaution, some of the Phygon will be found as phthalic acid. This effect can be minimized by carefully washing only the surfaces of fresh hard fruits (Miller, 1965). Data obtained by surface stripping are not acceptable because of the wide variety of fruits and vegetables now registered for use with Phygon. Corresponding photoconversion products can be minimized by careful handling and storage of the crop extracts in a cool dark place.

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