

1,3-Bis(3,4-dichlorophenyl)triazene from Propanil in Soils

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A major unknown product was isolated from ^{14}C -ring-labeled 3,4-dichloropropionanilide (propanil) in a Chikugo light clay soil. The identity of the unknown was established as 1,3-bis(3,4-dichlorophenyl)triazene by comparison of its physical and chemical properties with a synthetic sample. Infrared, ultraviolet, nuclear magnetic resonance, and mass spectra were determined. The triazene

probably arises by formation of an intermediate diazonium cation which subsequently couples with 3,4-dichloroaniline. It is proposed that soil nitrite initially reacts with 3,4-dichloroaniline to form the intermediate diazonium cation. Consequently soil nitrite content may determine the subsequent pathways of 3,4-dichloroaniline metabolism into various condensation products or formation of the triazene.

Recently a number of condensation products arising from 3,4-dichloroaniline have been reported in soils receiving high dosages of certain acylanilide herbicides. Bartha and Pramer (1967) showed that 3,4-dichloroaniline from 3,4-dichloropropionanilide (propanil) was converted to 3,3',4,4'-tetrachloroazobenzene (TCAB) in Nixon sandy loam soil. The formation of azobenzenes from anilines depended on the position of chlorine substituents as well as on a number of other factors (Bartha *et al.*, 1968). Condensation of three molecules of 3,4-dichloroaniline has now been reported by Rosen *et al.* (1969), and the implications of their findings have necessitated further study of the fate of aniline-based herbicides in soils.

We have demonstrated that acylated anilines in soils result from microbial conversion of the starting material 3,4-dichloroaniline (Plimmer and Kearney, 1969). Katz *et al.* (1969) examined the possibility that diazotization reactions in rumen fluid could convert an aniline into a reactive diazonium compound. Further reaction with another molecule of free aniline or a reactive substrate might afford an azo compound. Their findings were negative, but do not exclude the possibility that such reactions might occur under suitable environmental conditions. A study of ^{14}C -ring labeled propanil, incubated for 25 days in a number of Japanese soils (Chisaka and Kearney, 1970), provided evidence for yet another major product not previously reported in soils. The present paper describes the isolation, identification, and probable *in vivo* synthesis of this unknown compound in soils.

EXPERIMENTAL

Mass spectra were obtained with a Perkin Elmer Model GC 270 instrument or (high resolution) on a CEC Model 21-110 instrument. Infrared spectra were determined as KBr disks on Perkin Elmer Model 621 infrared spectrophotometer.

Isolation of 1,3-Bis(3,4-dichlorophenyl)triazene. A Japanese light clay (Chikugo) soil treated with ^{14}C -propanil uniformly ring labeled (0.77 μCi per 50 g of soil) and sufficient purified propanil to give a concentration of 850 ppm in soil was incubated for 25 days. A sample (100 g oven-dry basis) was extracted with 200 ml of acetone, as described by Chisaka and Kearney (1970). The acetone extract was filtered and its

volume reduced. The aqueous layer was extracted with benzene (three volumes of 25 ml). The benzene extract was dried (MgSO_4) and concentrated in a rotary evaporator.

The benzene solution was applied to one corner of a 20 cm $\times$ 20 cm thin-layer chromatographic plate coated with a 2 mm layer of silica gel GF (Merck). Benzene was used as the first developing solvent; the plate was then dried, rotated through 90° , and developed with Solvent A (hexane, benzene, acetone 7:3:1, v/v). Chromatograms were exposed to no-screen x-ray film for 10 days. A number of yellow spots visible on the silica plate corresponded in position to spots visible on the developed x-ray film. The most intensely radioactive spot (R_f in benzene 0.68; R_f in Solvent A 0.30) was selected for further examination.

The unidentified spot was scraped from the plate and extracted from the silica gel several times with a little absolute ethanol. The combined washings were filtered through diatomaceous earth and evaporated to dryness. The pale orange-yellow residue was submitted for further analyses by mass, nuclear magnetic resonance (nmr), ultraviolet, and infrared spectrometry.

Synthesis of 1,3-Bis(3,4-dichlorophenyl)triazene. The compound was synthesized by the method described in the literature for 1,3-bis(phenyl)triazene (diazoaminobenzene) (Vogel, 1956). 3,4-Dichloroaniline was used as starting material and quantities were varied accordingly. A yellow solid was collected and purified by treatment with charcoal and crystallization from petroleum ether b.p. $60\text{--}110^\circ\text{C}$, followed by recrystallization from hexane. It crystallized as pale-yellow needles, m.p. 153°C , showing a transition to rods at 145°C [Usui and Matsumura (1967) give m.p. 151°C]. The chromatographic and physical properties were identical with those of the compound isolated from soil. The molecular formula $\text{C}_{12}\text{H}_7\text{N}_3\text{Cl}_4$ was confirmed by high resolution mass spectrometry, as were the compositions of the fragment ions $\text{C}_{12}\text{H}_7\text{NCl}_4$ and $\text{C}_6\text{H}_5\text{NCl}_2$.

RESULTS AND DISCUSSION

In the preceding paper (Chisaka and Kearney, 1970) the techniques used for extracting soils and obtaining herbicide metabolites have been described. If individual products separated by thin-layer chromatography contained a significant amount of the ^{14}C -label, efforts were made to isolate and identify them. A compound isolated from a Japanese clay soil (Chikugo) contained more than 60% of the total extract-

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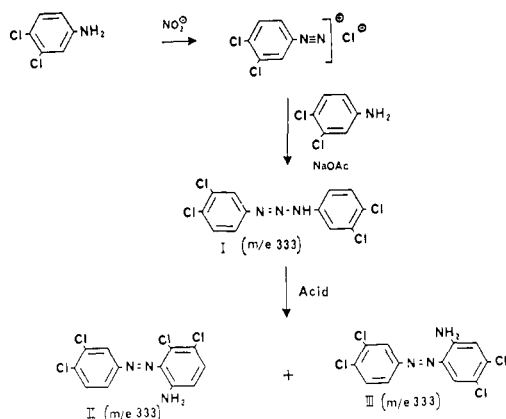


Figure 1. Formation and rearrangement of 1,3-bis(3,4-dichlorophenyl)triazene

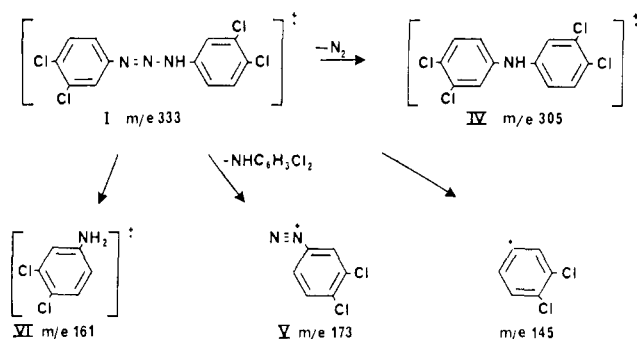


Figure 2a. Fragmentation of 1,3-bis(3,4-dichlorophenyl)triazene

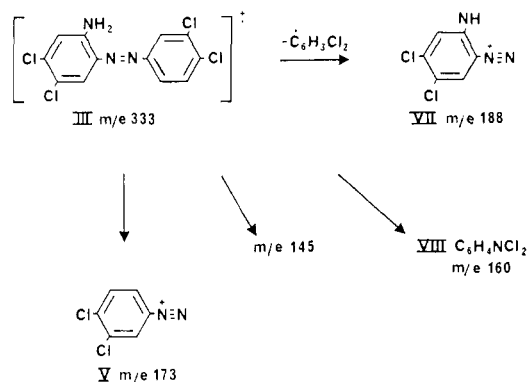


Figure 2b. Fragmentation of a tetrachloroaminobenzene (Katz *et al.*, 1969)

able radioactivity (25% of the amount initially added). On thin-layer chromatograms, the unknown compound appeared as a yellow spot, less mobile than 3,3',4,4'-tetrachloroazobenzene. As the compound was not sufficiently volatile for gas chromatography, we suspected that it might be 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene *m/e* 443, reported by Rosen *et al.* (1969). However, a sample examined by mass spectrometry had molecular ion at *m/e* 333. The ultraviolet spectrum in ethanol had maxima at 244, 305, and 363 nm, compared with 240, 322, and 443 nm for 3,3',4,4'-tetrachloroazobenzene, and with 238, 270, and 331 nm for 4,4'-dichloroazoxybenzene. High resolution mass spectrometry showed that the molecular ion of *m/e* 333 had the empirical formula $C_{12}H_7N_3Cl_4$. Fragment ions at *m/e* 305 and 161 had the formula $C_{12}H_7N^{\pm}Cl_4$ and $C_6H_5N^{\pm}Cl_2$, respectively.

This evidence eliminates possible oxygenated structures. The presence of three nitrogen atoms is in agreement with the inference that *m/e* 333, an odd numbered mass, is truly the molecular ion. The formula $C_{12}H_7N_3Cl_4$ represents several possibilities. Of these, a triazene (diazoaminobenzene) or an aminoazobenzene appear most likely. Katz *et al.* (1969) obtained isomeric aminoazobenzenes by reaction of 3,4-dichloroaniline with nitrous acid. Under the conditions used, the isomeric aminoazobenzenes are probably formed in this reaction (Figure 1). Katz *et al.* (1969) reported the mass spectra of two compounds *m/e* 333 which they proposed were 6-amino-2,3,3',4'-tetrachloroazobenzene (II) and 2-amino-3',4,4',5-tetrachloroazobenzene (III). The molecular weight and fragmentation pattern support the aminoazobenzene structures. However, fragmentation of (I) obtained from soil revealed ions at *m/e* 305 (IV) by loss of nitrogen and *m/e* 173 (V) by loss of $Cl_2C_6H_3NH$ (Figure 2a). A fragment at *m/e* 161 ($C_6H_5Cl_2N$) (VI) must arise by a rearrangement process. In contrast, Katz *et al.* (1969) reported a different fragmentation pathway. Their tetrachloroaminobenzenes did not appear to lose nitrogen, but fragmented by loss of $C_6H_5Cl_2$ to give an aminodiazonium ion *m/e* 188 (VII) (Figure 2b). The fragment *m/e* 173 (V) is common to both schemes. They did not observe a fragment of *m/e* 161 but an ion *m/e* 160 (VIII) was obtained, possibly by loss of nitrogen from the ion *m/e* 188.

The preparation of diazoaminobenzene (Vogel, 1956) involves the reaction of benzenediazonium chloride with aniline in the presence of sodium acetate. Diazoaminobenzene can be isolated and is stable, but may rearrange under acidic conditions. The reaction of 3,4-dichloroaniline under analogous conditions afforded 1,3-bis(3,4-dichlorophenyl)triazene as a yellow solid. The infrared spectrum of the authentic triazene was superimposable on that of the unknown compound from soil. A broad absorption in the infrared at 3420–3460 cm^{-1} may be ascribed to the NH stretching vibration (Nakanishi, 1962) of a secondary amine. A second weaker band might be observed in this region if a primary aromatic amino group were present (Dolinsky and Jones, 1954). The nmr spectrum was consistent with the allocated structure. The mass spectrum, ultraviolet absorption, and chromatographic behavior of the unknown compound were identical with those of authentic 1,3-bis(3,4-dichlorophenyl)triazene.

Transformations of pesticides in soils are the result of the interaction of a number of processes. Soil characteristics have been recognized as important factors in determining the quantitative relationships between the metabolites of a pesticide. On the other hand, qualitative relationships may be related to the microbial flora of the soil. The conversion of 3,4-dichloroaniline to a triazene probably requires the formation of an intermediate diazonium cation, which subsequently reacts with free 3,4-dichloroaniline. The diazonium cation may result from the reaction between dichloroaniline and soil nitrite. The formation of triazene would therefore depend on nitrite content or fertility status of the soil. In the previous paper, the detection of amounts of triazene in a soil which had previously yielded relatively large amounts of TCAB was reported. This observation suggests that if several crops of rice seedlings are returned to the soils between experiments, the nitrite level and consequently the nature of the terminal residues may be profoundly affected. Our findings therefore lead to the conclusion that the fertility status of the soil could affect the course of metabolism of certain pesticides.

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