

## Mixed Chloroazobenzene Formation in Soil

Condensation of 3-chloroaniline and 3,4-dichloroaniline to form 3,3',4'-trichloroazobenzene in addition to 3,3'-dichloroazobenzene and 3,3',4,4'-tetrachloroazobenzene (TCAB) has been found in

Nixon sandy loam soil. The mixed chloroazobenzene also arises during synthesis of TCAB by  $\text{LiAlH}_4$  reduction.

Several aniline herbicides yield azobenzenes as products in soil. Soil microorganisms are responsible for the transformation of 3',4'-dichloropropionilide (propanil) to 3,4-dichloroaniline and subsequently 3,3',4,4'-tetrachloroazobenzene (TCAB) (Bartha and Pramer, 1967). In 30 days, 46% of the aromatic moiety of propanil was recovered as TCAB in a Nixon sandy loam. In a subsequent study (Bartha *et al.*, 1968), all monochloro- and several dichloroanilines were converted to their corresponding dichloro- and tetrachloroazobenzenes. The isolated enzyme from *Pseudomonas striata* Chester can convert propanil to 3,4-dichloroaniline (Kearney, 1965). Peroxidase oxidation of some substituted anilines was investigated by Daniels and Saunders (1953). Only in the case of the chloro-substituted anilines was an appreciable amount of azobenzene obtained. Therefore, the number and nature of substituents in the aromatic ring greatly influence azobenzene formation.

In the present study, a mixture of 3-chloroaniline and 3,4-dichloroaniline was added to Nixon sandy loam soil to determine whether a trichloroazobenzene, in addition to the di- and tetrachloroazobenzenes, could possibly arise as a product of condensation.

### EXPERIMENTAL

3-Chloroaniline was purified by vacuum distillation. The 3,4-dichloroaniline was purified by charcoal treatment and several recrystallizations from petroleum ether. Both 3,3'-dichloro-

roazobenzene (DCAB) and 3,3',4,4'-tetrachloroazobenzene (TCAB) were synthesized by  $\text{LiAlH}_4$  reduction of *m*-chloronitrobenzene and 1,2-dichloro-3-nitrobenzene, respectively, as described by Corbett and Holt (1963). The purity of the reaction product from each preparation was established by gas chromatography and mass spectral analysis.

Four 50-gram samples of Nixon sandy loam were treated in the following manner: sample A received 0.1 gram of 3-chloroaniline; sample B, 0.1 gram of 3,4-dichloroaniline; and sample C, 0.05 gram of 3-chloroaniline plus 0.05 gram of 3,4-dichloroaniline. A blank sample containing no chloroaniline was also included. The chloroanilines were added to the soil in acetone solutions and the acetone was allowed to evaporate. The samples were mixed and adjusted to approximately 60% field capacity. Following incubation for 21 days at 30° C. in an incubator, each soil was extracted with 100 ml. of redistilled hexane and evaporated to a final volume of 10 ml. The 3 samples are subsequently referred to as extracts A, B, and C.

The chloroazobenzenes were examined by gas-liquid chromatography using a Research Specialties Model 600 with a flame detector and column (6 feet  $\times$  1/4 inch) packed with 5% SE30 on Chromosorb W (60- to 80-mesh) isothermally at 238°.

Mass spectral analysis was performed with a Perkin-Elmer Model 270 combination gas chromatograph-mass spectrometer. The GC column used was a 50-foot surface-coated

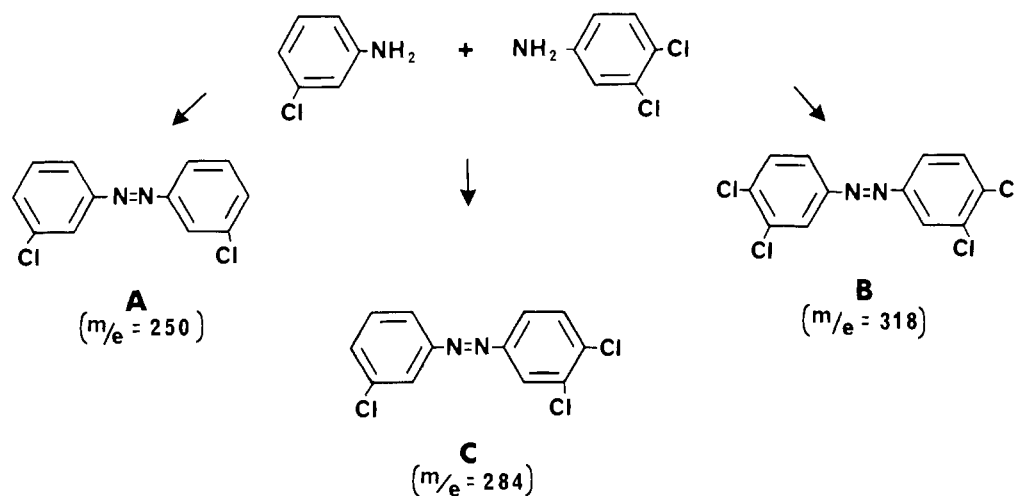


Figure 1. Proposed pathways for formation of TCAB, DCAB, and 3,3',4-trichloroazobenzene in soil

open tubular column of 0.02-inch internal diameter coated with SE30 on Chromosorb W. Retention order was the same as that from the packed column.

#### RESULTS AND DISCUSSION

The soil extracts were examined by gas chromatography. Extract A showed one major peak as did extract B, whereas extract C showed three peaks. Combination gas chromatography-mass spectrometry was used to identify the compounds: tentative indications were provided by retention times. Extract A contained only DCAB, B contained only TCAB, and extract C contained DCAB, TCAB, and 3,3',4-trichloroazobenzene. (Figure 1). The molecular ions of these azobenzenes at  $m/e$  250, 318, and 284, respectively, showed peaks at higher mass having the correct intensity ratios for the expected number of chlorine atoms due to the presence of  $^{37}\text{Cl}$ . In the case of DCAB and TCAB fragmentation patterns were compared with those of authentic specimens. 3,3',4-Trichloroazobenzene showed a fragmentation pattern typical of the chlorinated azobenzenes. Gas chromatography of an extract of the control sample afforded no peaks in the region of interest. The formation of TCAB and DCAB in soils from the individual chloroanilines has been reported (Bartha *et al.*, 1968), but we consider it noteworthy that condensation of two different anilines may occur to give a mixed azobenzene. The detection of 3,3',4-trichloroazobenzene suggests that mixed azobenzenes may be formed in soils receiving one or more aniline herbicides.

Further condensation reactions of aniline in soil probably occur as thin-layer chromatography of the three soil extracts revealed at least 10 colored compounds not present in the control. TLC was performed on silica gel coated plates developed with benzene, hexane, ethyl acetate (3:7:1). In the case of A and B, these probably represent self-condensation products, whereas mixed products may occur in the extract

C. The complexity of the mixtures, however, makes comparison difficult.

Synthesis of the reference compounds by lithium aluminum hydride reduction of the corresponding chlorinated nitrobenzenes was accompanied by reductive dechlorination. Tetrachloroazobenzene was prepared and, following column chromatography, contained traces of trichloroazobenzene and dichloroazobenzene on examination by GC-mass spectrometry. Dechlorination of chlorinated nitroarenes by lithium aluminum hydride has been previously noted where chlorine is situated ortho to the nitro group and to a second chlorine atom (Linke *et al.*, 1968).

**Note.** While this work was in progress, a similar report on asymmetric azobenzene formation appeared in the literature, Bordeleau, L. M., Linke, H. A. B., Bartha, R., *Bacteriol. Proc.* A21, 1969.

#### LITERATURE CITED

- Bartha, R., Pramer, D., *Science* **156**, 1617 (1967).  
Bartha, R., Linke, H. A., Pramer, D., **161**, 582 (1968).  
Corbett, J. F., Holt, P. F., *J. Chem. Soc.*, **1963**, 2385.  
Daniels, D. G. H., Saunders, B. C., *J. Chem. Soc.* **1953**, 833.  
Kearney, P. C., *J. AGR. FOOD CHEM.* **13**, 561 (1965).  
Linke, A. B., Bartha, R., Pramer, D., *Naturwiss.* **55**, 444 (1968).

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