Photolysis of Trifluralin: Characterization of Azobenzene and Azoxybenzene Photodegradation Products

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Three azoxybenzene derivatives, *N*-propyl-2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*p*-toluidine) (I), 2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine) (II), and 2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*p*-toluidine) (V), and two azobenzene derivatives, *N*-propyl-2,2′-azobis(α,α,α-trifluoro-6-nitro-*p*-toluidine) (III) and 2,2′-azobis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine) (IV), have been identified as products from UV photolysis of trifluralin in benzene solutions. Purification of individual products was performed by column and thin-layer chromatography. Infrared, nuclear magnetic resonance, and mass spectral data of these products are presented and discussed. Chemical reduction of the azoxybenzene I yielded numerous products, including the corresponding azobenzene III and hydrazo derivative [1,2-bis(α,α,α-trifluoro-2-amino-3-nitrotolyl)hydrazine]. Also II was reduced to IV. This work confirms and extends other workers' indications that azoxybenzene derivatives are formed by the photolysis of trifluralin and demonstrates the formation of azobenzenes.

Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) (Figure 1a) is a widely used selective preemergence herbicide that has been demonstrated to undergo extensive photodegradation resulting in diverse chemical classes of products. The degradation of dinitroaniline herbicides, including trifluralin, has been reviewed (Probst et al., 1975). Among the photodegradation products of trifluralin that have been identified are benzimidazoles, benzimidazole oxides, benzimidazolines, and species arising from reduction of the nitro groups and dealkylation of the tertiary amine (Leitis and Crosby, 1974). In addition, azobenzenes and azoxybenzenes were suspected photodegradation products arising from the dimerization of two modified trifluralin molecules and preliminary spectroscopic data supporting these structural assignments were presented in two theses (Leitis, 1973; Markle, 1974). After the completion of the studies reported here, definitive results were published by Golab and co-workers (1979). Degradation of ^{14}C-labeled trifluralin, which was applied to soil in field studies, resulted in the formation of at least 28 compounds which have been identified by thin-layer chromatography (TLC) and in some cases mass spectroscopy (Golab and Occolowitz, 1979). These compounds included members of all the chemical classes mentioned above in addition to several others.

In this study we have been concerned with the direct photochemical production of azobenzenes and azoxybenzenes from trifluralin and the characterization of these compounds by spectroscopic analysis and chemical modification methods. While the work of Golab et al. (1979) indicates the formation of azobenzenes and azoxybenzenes from trifluralin in soil, it leaves unanswered the question as to whether they were formed via biological or abiological pathways. This work demonstrates that they can be formed by a purely photochemical mechanism involving no biological intermediaries.

MATERIALS AND METHODS

Technical trifluralin (a gift from Eli Lilly and Co., Indianapolis, IN) was recrystallized from hexane (mp 47–47.5 °C) until homogeneous by TLC. α,α,α-Trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine (VII, also a gift from Eli Lilly and Co.) was used as a TLC standard as received. Stannous chloride ($SnCl_2 \cdot 2H_2O$) and sodium borohydride ($NaBH_4$) were used in the commercially supplied form. Preparative TLC was performed with 1.0 mm thick layers of silica gel G (type 60, EM Reagents, Darmstadt, Germany). Analytical TLC was performed with either Baker-flex silica gel IB (J. T. Baker Chemical Co., Phillipsburg, NJ) or Eastman chromagram sheets (13179 silica gel without fluorescent indicator, Eastman Kodak Co., Rochester, NY). All solvents used were reagent grade or better and were used as supplied without further purification. Hexane as used in this paper refers to a mixture of isomers of hexane as supplied by Eastman Kodak Co. (Rochester, NY). Isoamyl alcohol (2-methyl-1-butanol; Allied Chemical, New York, NY) was distilled once before use.

Hydrogen nuclear magnetic resonance (NMR) spectra were obtained from $CDCl_3$ solution, using a 100-MHz Varian XL-100 nuclear magnetic resonance spectrometer equipped with a Varian 620 computer having 16K memory and producing 8192 data points for multiple acquisition NMR spectra (Varian Associates, Palo Alto, CA). IR spectra were obtained with a Beckman Acculab 6 spectrophotometer with KBr disks of about 1% sample concentration. EI mass spectra were determined on a Kratos/AEI MS 50 ultrahigh-resolution mass spectrometer equipped with a Kratos DS-50 data system. A typical set of conditions used was as follows: cage temperature, 150 °C; pressure, 10^{-6} torr; probe temperature, 75 °C; 70 eV. Values of m/z which were taken from high-resolution mass spectra are reported to 0.0001 mass unit and those from low-resolution mass spectra obtained with data system assistance to 0.01 mass unit. Some mass spectra were recorded on a strip chart and the m/z values from such spectra are reported to 1 mass unit. For some compounds, high m/z peaks could be observed only on the low-resolution mass spectra. As a result, the peaks reported for some compounds were obtained from two different spectra, one high resolution and one low resolution. Elemental analyses were performed by Huffman Laboratories, Inc., Wheatridge, CO. Because fluorine interferes with the oxygen determination, oxygen was not determined.

Photolysis of Trifluralin. Twenty grams of trifluralin was dissolved in 1800 mL of benzene and placed in a 2-L Pyrex photoreaction vessel fitted with a nitrogen gas sparger, water-cooled reflux condenser, and water-cooled quartz immersion well. The solution was sparged with nitrogen for an hour before irradiation was started. A medium pressure mercury vapor lamp (Hanovia Hg arc, 450 W) placed inside the immersion well was used to irradiate the stirred solution for 24 h. The reaction mixture was concentrated to a volume of about 20 mL on a flash evaporator and applied to a Florisil (60–100 mesh) column,

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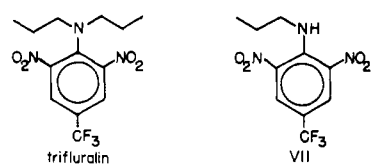

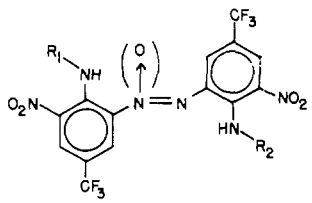

COMPOUND	AZO or AZOXY	R_1	R_2
I	azoxy	C_3H_7	H
II	azoxy	C_3H_7	C_3H_7
III	azo	C_3H_7	H
IV	azo	C_3H_7	C_3H_7
V	azoxy	H	H

b

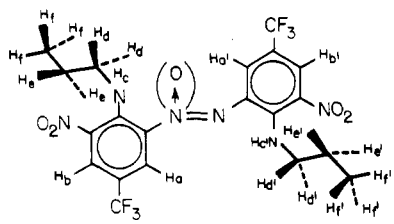

Figure 1. (a) Structures of trifluralin and its photodegradation products identified in this study. (b) Subscripts used to assign hydrogen NMR signals.

3.0 cm diameter × 800 cm length, followed by elution with 1000 mL of hexane. A yellow band (A) was removed from the column, leaving a dark band at the top of the column. Elution with 0.5% acetone in hexane produced a visually distinguishable, light-yellow fraction (B). A third, red band (C) was removed from the column, using about 1000 mL of 2% acetone in hexane. A fourth fraction (D) was removed from the column with about 1500 mL of 5% acetone in hexane solution. A 15% acetone in hexane solution removed a number of colored compounds from the column as one band (E).

Upon evaporation of the solvent of band A, a yellow oil formed which was crystallized from hexane to form yellow crystals and was shown to be trifluralin by use of TLC and IR spectroscopy. B was concentrated and applied to preparative TLC plates and was developed in hexane–benzene (33:67). The major band, light yellow, of several bands observed (R_f 0.75) was scraped from the plate and extracted with diethyl ether, concentrated, and applied to a second plate and developed in hexane–acetone (90:10). One major band was produced (R_f 0.63) and identified as VII, a compound previously reported by several workers (Probst and Tepe, 1969; Harrison and Anderson, 1970; Crosby and Leitis, 1973). Fractions C and D upon concentration in a flash evaporator to a volume of about 5 mL, followed by evaporation under a stream of nitrogen, produced crystals, which were eventually identified as II and I, respectively. Both I and II were recrystallized twice from chloroform–isooctane. Fraction E was subjected to preparative TLC and developed with benzene–petroleum ether (50:50). The purple band showing the greatest migration (R_f 0.56) was extracted with acetone, and evaporation of the solvent under a stream of nitrogen produced purple crystals. These were recrystallized twice from hexane–acetone, yielding a compound identified as IV. An intense yellow band (R_f 0.20) was extracted with acetone from the same TLC plates as E and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 1.0 mL of acetone and subjected to further preparative TLC developed several times with $CHCl_3$. The major dark yellow-orange band was extracted and crystallized twice from a hexane–acetone solution, yielding V.

Another compound of interest was more easily purified by an alternate procedure. After elution of the Florisil column with hexane, the column was stripped by using CH_3OH–$CHCl_3$ (50:50). This methanol–chloroform fraction was then concentrated and subjected to preparative TLC developed with hexane which produced five clearly distinguishable bands. One band (R_f 0.05) was extracted with methanol–chloroform and rechromatographed again using hexane–acetone (50:50). Upon concentration and crystallization from acetone and hexane, purple needles were obtained and identified as III.

Two reagents were used to reduce the azoxybenzenes to azobenzenes and a hydrazobenzene. The use of $NaBH_4$ to reduce polysubstituted azoxybenzenes to azobenzenes has been reviewed by Newbold (1975) and stannous chloride–hydrochloric acid has been reported as an effective reducing agent of substituted azoxybenzenes (Newbold, 1965).

$SnCl_2$·HCl Reduction of I. A solution of 0.27 g of $SnCl_2 \cdot 2H_2O$ (1.21×10^{-3} mol) and 0.20 mL of concentrated HCl (1.21×10^{-3} mol) in 15 mL of distilled absolute ethanol was added dropwise to a refluxing solution of 100 mg of I (2.04×10^{-4} mol) in 15 mL of absolute ethanol over a period of approximately half an hour. After cooling to room temperature, the solution was neutralized by addition of dilute $NaHCO_3$ to a total volume of 100 mL. This aqueous solution was extracted with three 50-mL portions of diethyl ether which effectively removed all colored products from the aqueous solution. The resulting red solution was dried over anhydrous $MgSO_4$ overnight. After filtering and reduction of volume, it was subjected to preparative TLC developed with hexane–benzene (50:50).

$NaBH_4$ Reduction of I. A solution of 10 mg of $NaBH_4$ (2.65×10^{-4} mol) in 10 mL of absolute ethanol was added dropwise to a refluxing solution of 25 mg of I (5.0×10^{-5} mol) in 25 mL of absolute ethanol. The solution darkened and was allowed to reflux an additional 2 h. After cooling to room temperature and diluting with 100 mL of distilled water, the products were extracted with three 50-mL portions of diethyl ether. Preparative TLC of the reaction mixture was developed with hexane–benzene (50:50).

$SnCl_2$·HCl Reduction of II. A solution of 83 mg of $SnCl_2 \cdot 2H_2O$ (3.71×10^{-4} mol) and 0.31 mL of concentrated HCl (3.71×10^{-4} mol) in 25 mL of absolute ethanol was added dropwise to a refluxing solution of 20 mg of II (3.71×10^{-5} mol) in 25 mL of absolute ethanol over a period of about 0.5 h. The resulting solution was allowed to reflux for an additional half hour. The reaction mixture was diluted with dilute $NaHCO_3$, extracted, and dried as described above. TLC developed with petroleum ether–benzene (75:25) produced several bands.

$NaBH_4$ Reduction of II. A solution of 8 mg of $NaBH_4$ (2.11×10^{-4} mol) in 12 mL of absolute ethanol was added dropwise over a period of about 30 min to a refluxing solution of 16 mg of II (2.97×10^{-5} mol) in 25 mL of absolute ethanol, followed by 1 h of reflux. During this time the reaction mixture changed from orange to dark brown. After cooling, 100 mL of distilled water was added

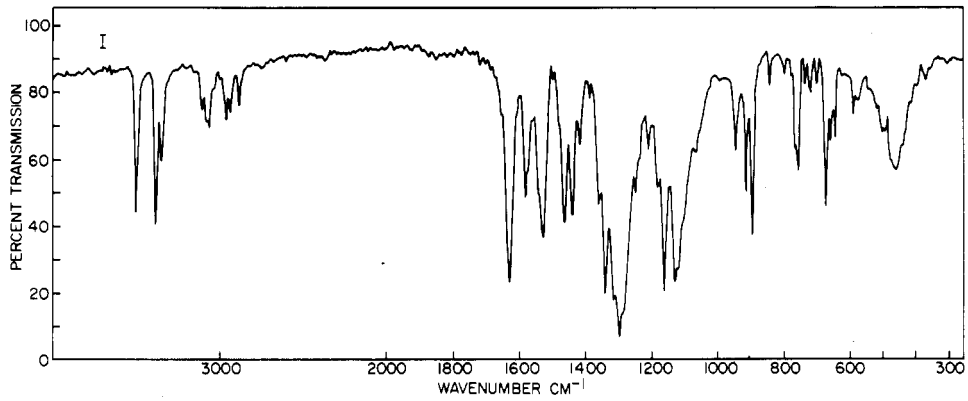

Figure 2. Infrared spectra of azobenzene and azoxybenzene photodegradation products of trifluralin and the hydrazo compound obtained by $NaBH_4$ reduction of I; azoxy, I.

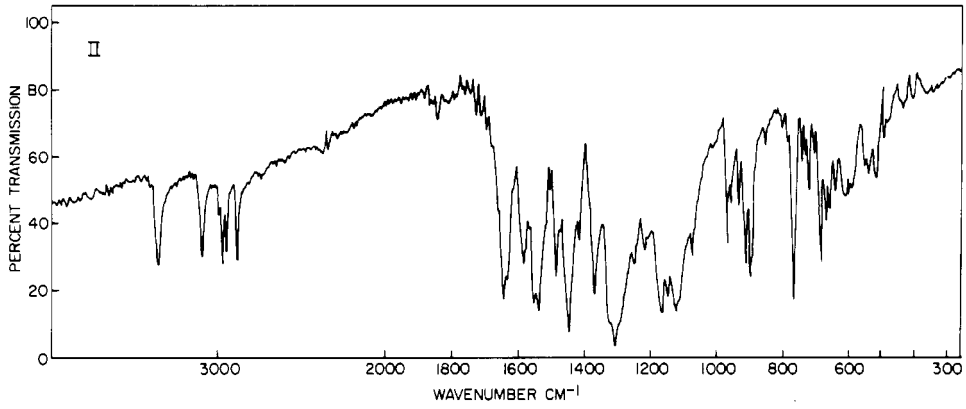

Figure 3. See Figure 2 caption; azoxy, II.

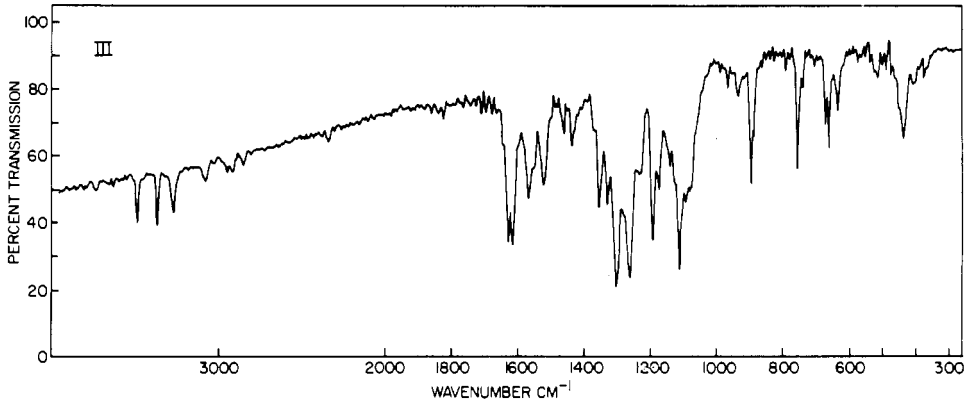

Figure 4. See Figure 2 caption; azo, III.

to the ethanol solution and it was extracted with two 50-mL portions of diethyl ether. The ether extract was dried over $MgSO_4$ for 12 h, evaporated to a volume of 2 mL under a stream of nitrogen, and subjected to preparative TLC developed with $CHCl_3$.

Thermal Decomposition of I. A few milligrams of I was dissolved in 50 mL of isoamyl alcohol and heated to reflux for about 1 h. The solution gradually darkened. The solution was concentrated on a flash evaporator, and the residue was subjected to preparative TLC developed in $CHCl_3$.

RESULTS AND DISCUSSION

Crystals of I from band D [*N*-propyl-2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*p*-toluidine)] (Figure 1a) were obtained as yellow needles, mp 136.5–137.5 °C. The 20 g of trifluralin yielded 265 mg of I (1.3%) and represented 6.6% of the photodegradation products. Calculated for $C_{17}H_{14}F_6N_6O_5$: C, 41.14; H, 2.84; F, 22.87; N, 16.93; O, 16.12. Found: C, 41.04; H, 3.05; F, 22.80; N, 16.63.

The IR spectrum of I (Figures 2–7) strongly suggested the presence of the following functional groups: primary amine (3500, 3400 cm^{-1}), secondary amine (3350 cm^{-1}), aromatic nitro (1535, 1300, 890 cm^{-1}), and trifluoromethyl (1130 cm^{-1}). The assignment of the azoxy group absorptions was less obvious. It is known that an azoxy group produces two absorption bands: 1480–1450 and 1335–1315 cm^{-1} (Colthup et al., 1975). The first is very near the 1500 cm^{-1} aromatic band and the methylene scissoring band, while the second is in the same region as a nitro band and the C–N stretch of aromatic amines (1340–1270 cm^{-1}). All of these bands, in addition to the azoxy bands, should be represented by I. The situation is further complicated by the fact that the nitro group is known to be able to double its absorption bands in compounds with multiple nitro groups when the groups are noncoplanar with the aromatic ring. Stereochemical crowding by adjacent groups can cause twisting of the nitro groups which results in a de-

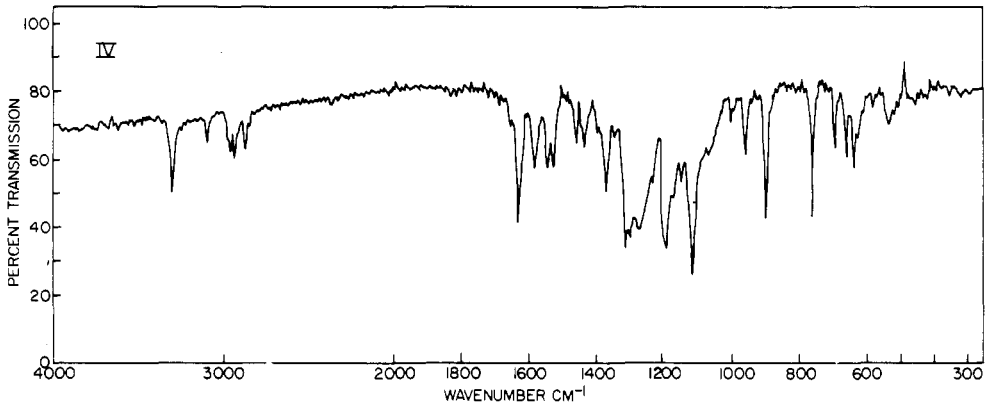

Figure 5. See Figure 2 caption; azo, IV.

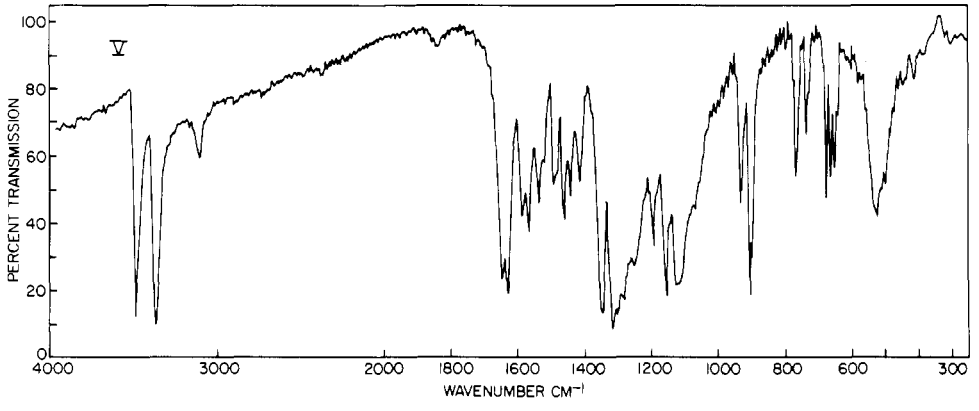

Figure 6. See Figure 2 caption; azoxy, V.

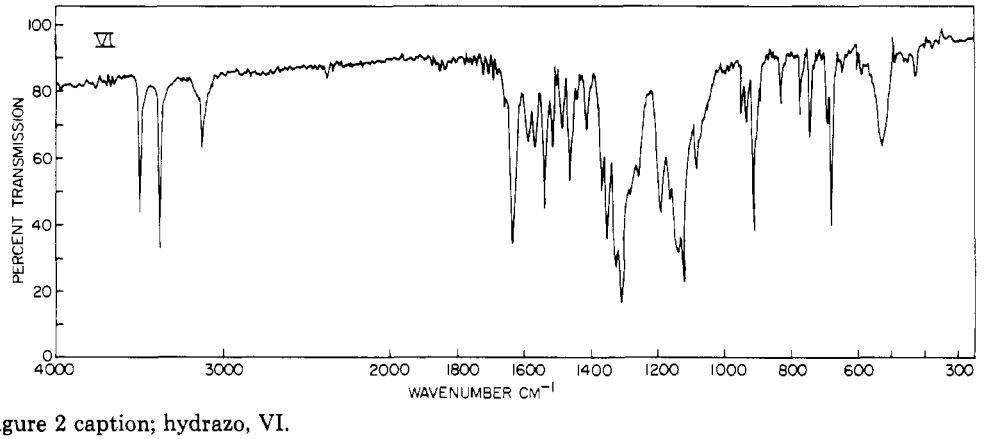

Figure 7. See Figure 2 caption; hydrazo, VI.

crease in the degree of aromatic conjugation, thus making a second higher energy transition possible (Bellamy, 1958). Examination of space filling models of I indicated that such a condition is likely in this compound. Comparison of IR spectra of similar compounds, particularly trifluralin and the azobenzene III, indicated that the absorption band at 1460 cm^{-1} could be assigned to the azoxy group with reasonable certainty.

The mass spectra fragmentation patterns observed were not entirely consistent with those described for less highly substituted azobenzenes and azoxybenzenes, which have been reported to undergo rearrangements and cleavage primarily between an azoxy or azo nitrogen and the ring carbon (Tam, 1975) rather than fission between the two azoxy or azo nitrogens as seen by Golab and Occolowitz (1979) and in this study.

The mass spectrum of I indicated that this compound is identical with a compound recently reported by Golab and Occolowitz (1979). The only noticeable difference between the two spectra was the relative intensities of some major peaks. The molecular ion was not always observed but could be detected in some spectra. The most intense peaks were at m/z 220.0328 and m/z 260.0655. A small peak at m/z 276.0567 indicated that the azoxy oxygen is located on the nitrogen attached to the same ring as the *N*-propyl group. These three peaks strongly suggest that the major fragmentation pathway involves cleavage between the azoxy nitrogens. The other peaks in the mass spectrum are consistent with further cleavage of these major fragments.

The NMR spectrum of I (Figures 8–13) showed a pattern consistent with an *N*-propyl group with a coupling of the amine hydrogen with the α-methylene hydrogens of the propyl group: δ 3.28 (qd, J = 6 Hz, H_d), 1.75 (s, J = 6 Hz, H_e), 1.0 (t, J = 6 Hz, H_c). Impurities, producing peaks at δ 1.30 and 1.60, were probably due to hydrocarbons or perhaps condensation products of the compounds being studied and acetone. The aromatic region

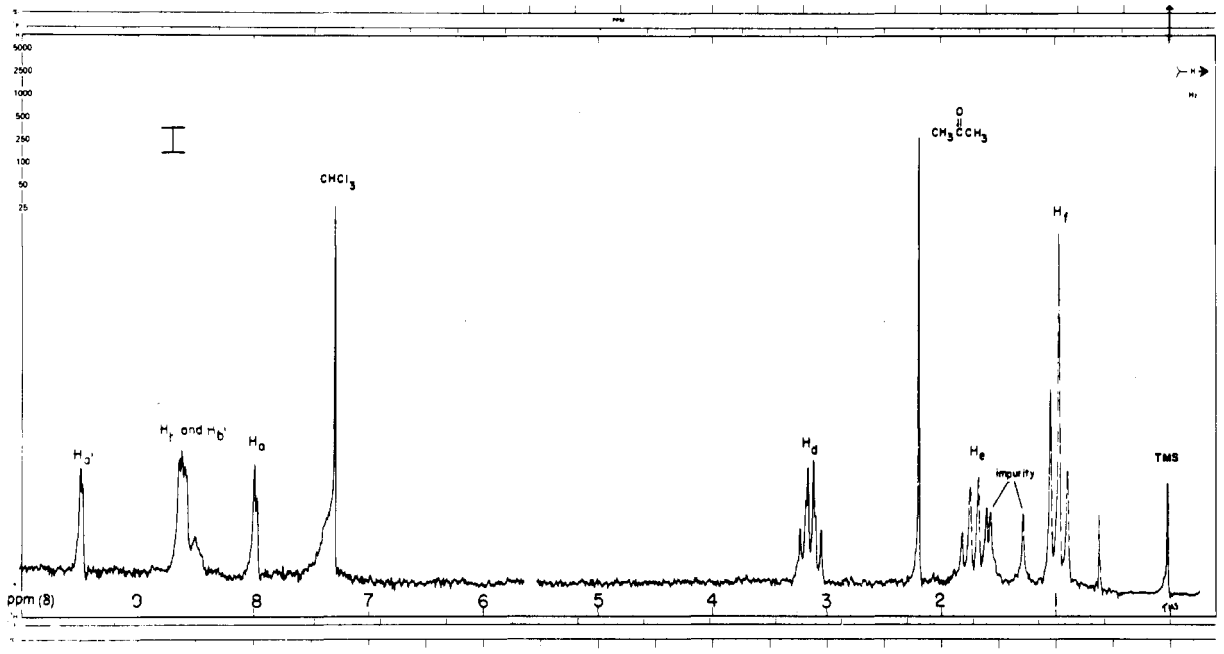

Figure 8. Hydrogen NMR spectra of the azobenzene and azoxybenzene photodegradation products of trifluralin discussed in this study; I.

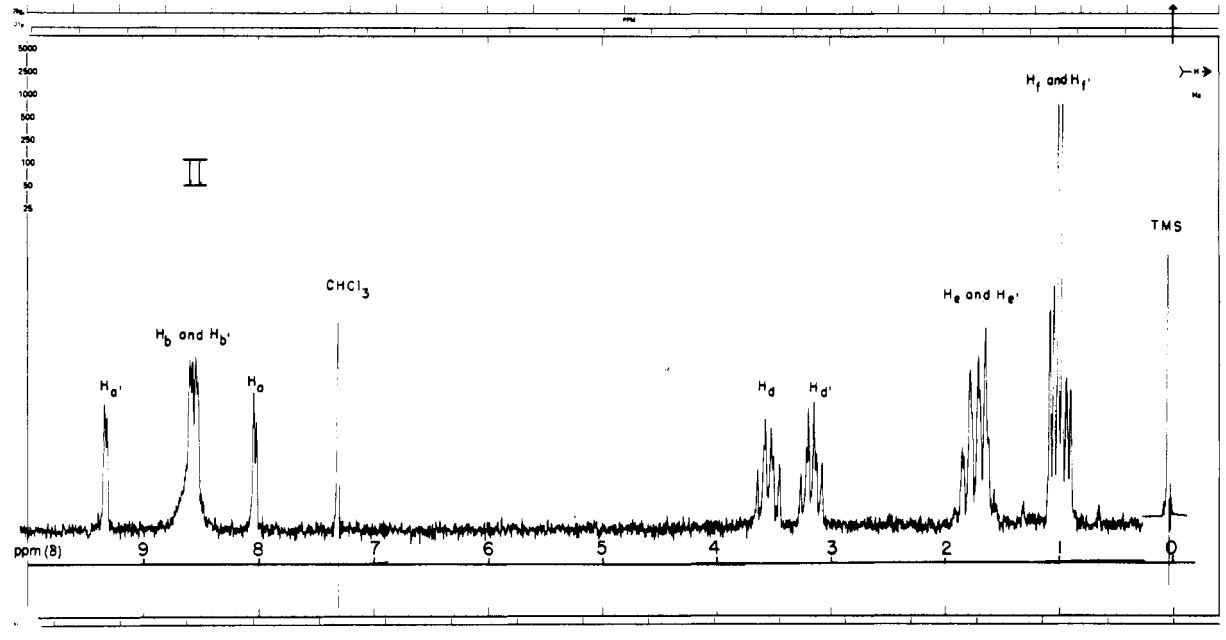

Figure 9. See Figure 8 caption; II.

of the spectrum showed three well separated sharp signals arising from the aromatic protons and two broad peaks ascribed to the amine protons. Compound I has four nonequivalent aromatic protons but both protons para to the azoxy group (H_b, H_b') would be expected to have nearly the same chemical shift. The signal at δ 8.63 appeared to be two closely spaced signals with an area approximately double that of the other sharp aromatic signals. Because of stereochemical constraints, I probably is in the cis conformation. As a result, the hydrogen ortho to the azoxy group and on the opposite ring would be in a position to be further deshielded by the azoxy oxygen. The signal at δ 9.55 could be assigned to this hydrogen (H_a'). The remaining aromatic hydrogen (H_a) was assigned the peak at δ 8.05. These peaks were split with a coupling constant, $J \simeq 2$ Hz, which was consistent with coupling to the adjacent trifluoromethyl groups (Leyden and Cox, 1977). Addition of D_2O to the sample tube eliminated the broad amine hydrogen peaks, and the quartet at approximately δ 3.2 (H_d) was simplified to a triplet. This behavior was consistent with deuterium exchange of the amine hydrogens.

The preparative TLC of the $SnCl_2$·HCl reduction products of I produced many colored bands. The band showing the greatest migration (R_f 0.21) was an intense purple color. (In TLC plates with less concentrated bands, this band appeared to be red rather than purple.) Extraction, concentration, and crystallization from $CHCl_3$–isooctane produced 3.5 mg (3.5% yield) of purple crystals. TLC of this compound in several solvent systems and its IR spectrum proved it to be identical with the azobenzene III, which had been isolated from the original photolysis mixture. Unreacted I was also isolated from the reaction mixture. Preparative TLC of the $NaBH_4$ reduction products of I showed three major colored bands in addition to many less intense bands. A red band showing the

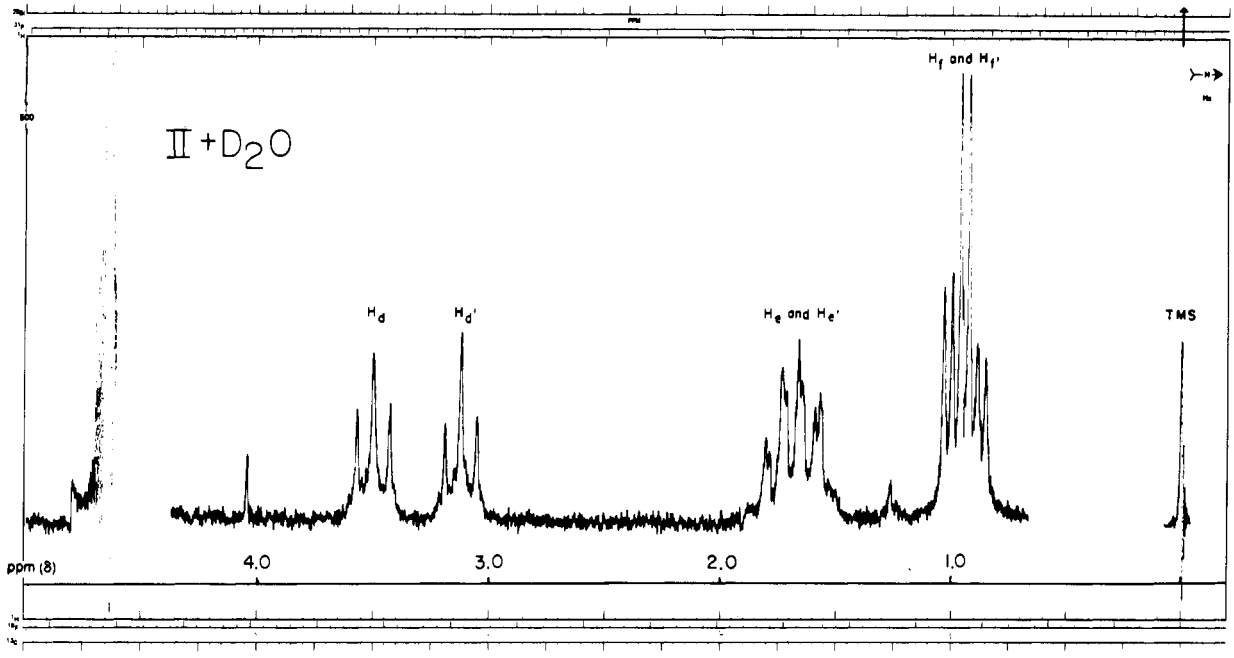

Figure 10. See Figure 8 caption; II + D_2O.

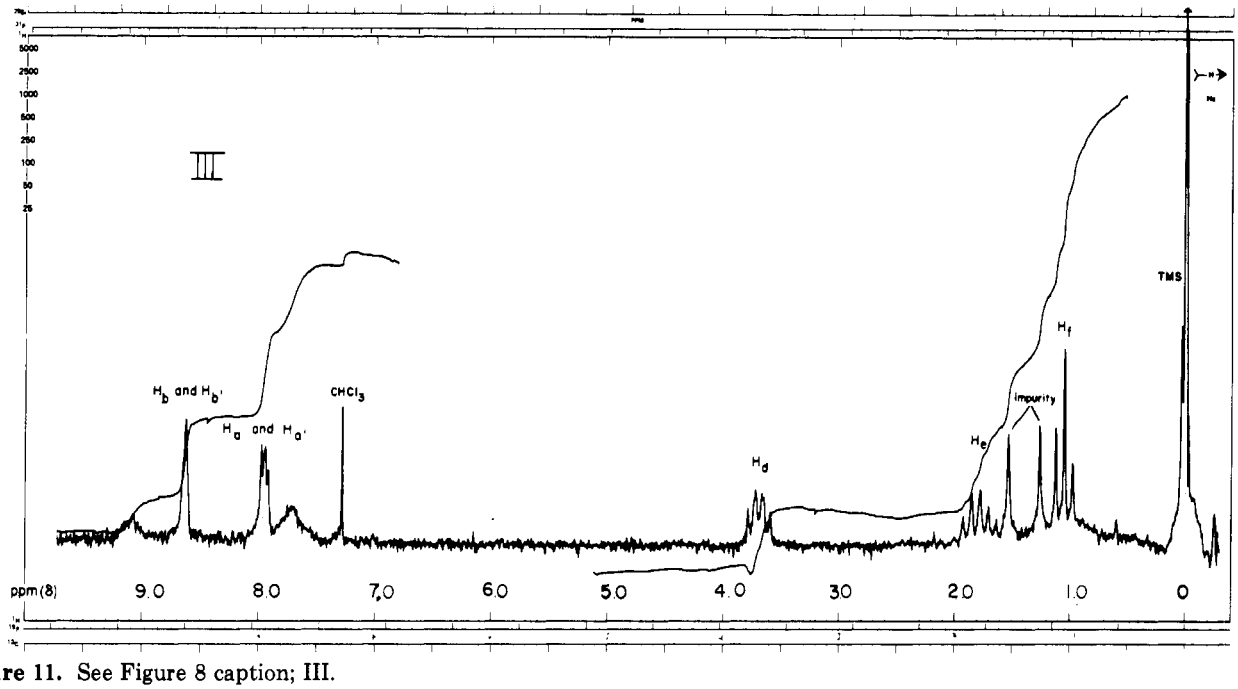

Figure 11. See Figure 8 caption; III.

greatest migration (R_f 0.21) produced a compound which had R_f values in several solvent systems identical with III, and its IR spectrum was the same as that of III. A dark-yellow band (R_f 0.13) was shown to be unreacted I by use of TLC and IR spectroscopy. A third major light-yellow band (R_f 0.07) was identified as VI, which was not present in the photolysis mixture. The reaction mixture resulting from the thermal decomposition of I produced only two bands of any intensity when subjected to TLC. A faint-red band immediately above a yellow band proved to be III by use of TLC and IR spectroscopy. The yellow band was shown to be I by using the same methods. These reactions are summarized in Figure 15.

Compound II from band C [2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)] (Figure 1) was obtained as orange, dense prisms (mp 140–141 °C). Twenty grams of trifluralin yielded 250 mg of II (1.25%) and represented 6.25% of the photodegradation products. Calculated for $C_{20}H_{20}F_6N_6O_5$: C, 44.62; H, 3.74; F, 21.17; N, 15.61; O, 14.86. Found: C, 44.73; H, 4.01; F, 20.38; N, 15.57.

The IR spectrum of II (Figure 3) showed many absorption bands in common with I but several significant differences were evident. Only one amine absorption band was present (3360 cm^{-1}), indicating the presence of only secondary amines in II. The aromatic ring bending or C=C stretching band at 1640 cm^{-1} had some doublet character, which was not unusual (Silverstein and Bassler, 1967). The lower frequency nitro band appeared to be doubled (1535, 1545 cm^{-1}) and the azoxy absorption at 1445 cm^{-1} was relatively more intense than for I.

The mass spectrum of II was similar to that reported previously for the same compound isolated from soil treated with trifluralin (Golab and Occolowitz, 1979), except that the molecular ion was not evident in our spectrum. The highest mass peaks we observed were m/z

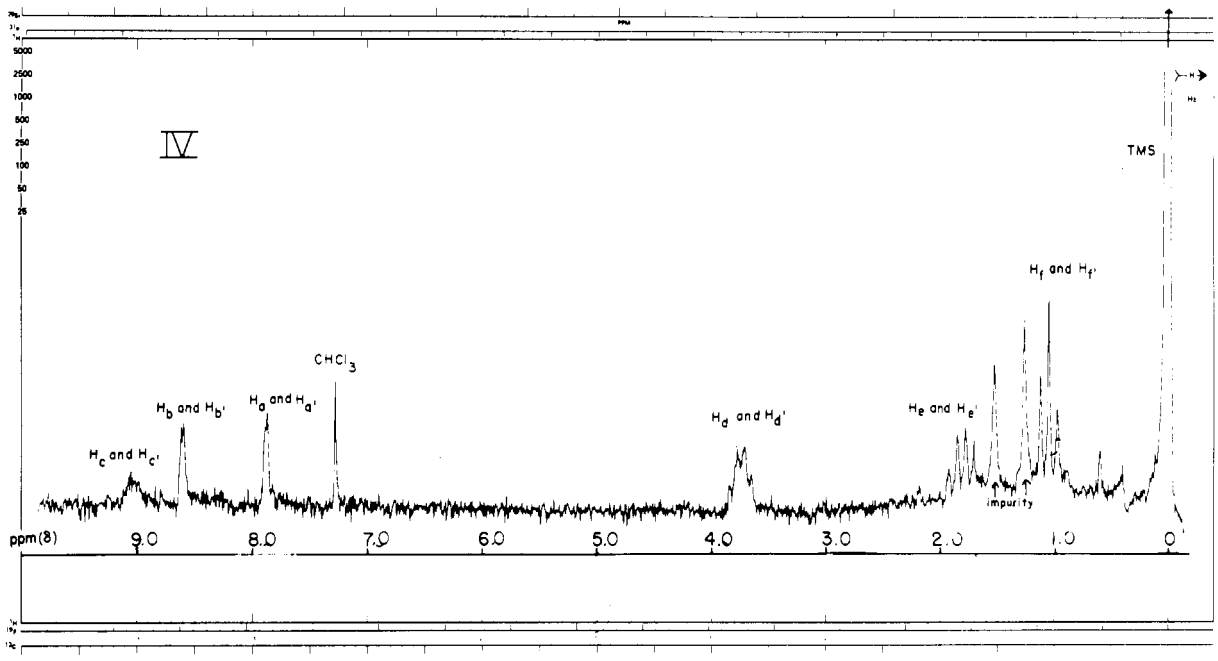

Figure 12. See Figure 8 caption; IV.

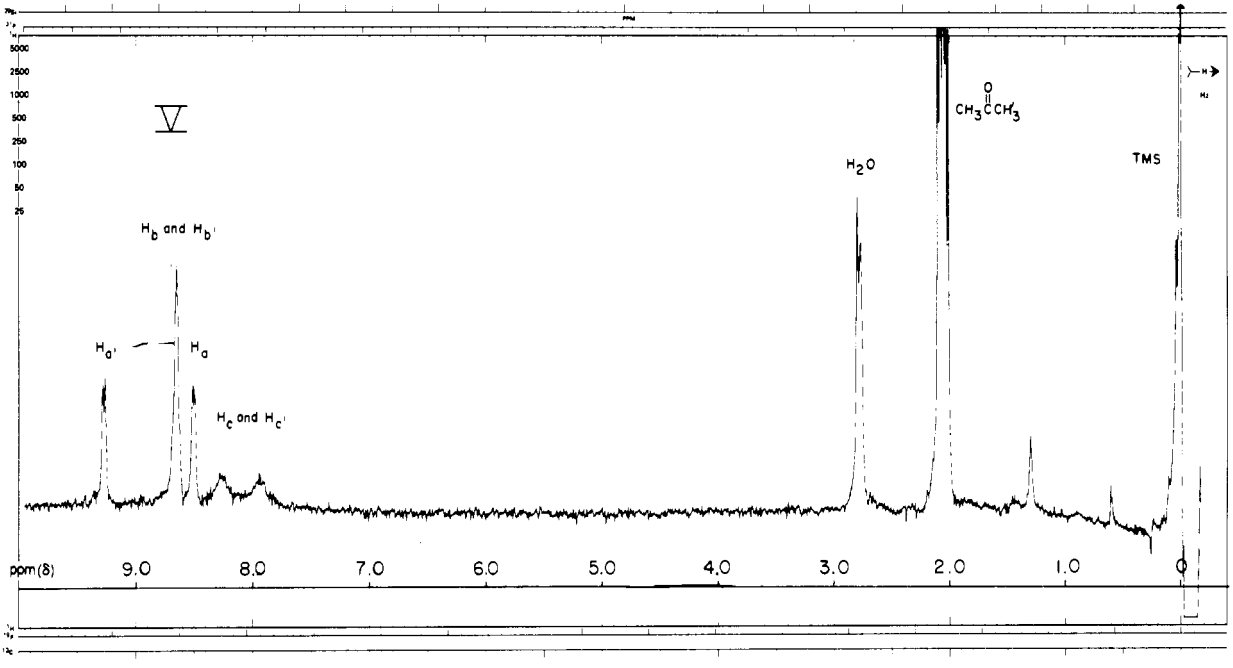

Figure 13. See Figure 8 caption; V.

520.15 and 519.15, which can be ascribed to loss of F and loss of O, respectively. The base peak was at m/z 232.0326. The fragmentation pattern of this compound was parallel to that of I, with the highest reasonably intense peak being at m/z 260.0636. This peak and a small peak at m/z 276.06 indicated that II also underwent fragmentation between the two azoxy nitrogens, followed by further cleavage of the resulting fragments.

The NMR spectrum of II (Figure 9) showed a very similar pattern in the aromatic region as I. However, II showed no peaks attributable to amine hydrogens. The signals at high field showed an interesting variation from III. Two sets of quartets due to the α-methylene groups coupled to the amine hydrogen were produced (δ 2.50 and 3.10). The β-methylene hydrogens (H_e, H_e') produced a sextet (δ 1.70) with marked side peaks. Two nearly superimposed triplets (δ 0.95) could be ascribed to the two methyl groups (H_f, H_f') of the *N*-propyl groups. The doubling of the propyl pattern may be easily explained by the asymmetry conferred on II by the azoxy group. Examination of space filling molecular models of II indicated the molecule to be constrained in the trans conformation. When arranged in the configuration showing the least stereochemical strain, the α-methylene hydrogens of the propyl group on the same side of the molecule as the azoxy oxygen were in a position to be further deshielded by the azoxy oxygen. It therefore appeared reasonable that the quartet at δ 3.50 was produced by this α-methylene group (H_d) and the quartet at δ 3.15 was due to the other α-methylene groups (H_d'). Deuterium–hydrogen exchange, using D_2O, reduced both quartets to triplets (δ 3.10, H_d'; 2.50, H_d) and made no significant changes in the spectrum (Figure 10).

Preparative TLC of the $SnCl_2$·HCl reduction of II

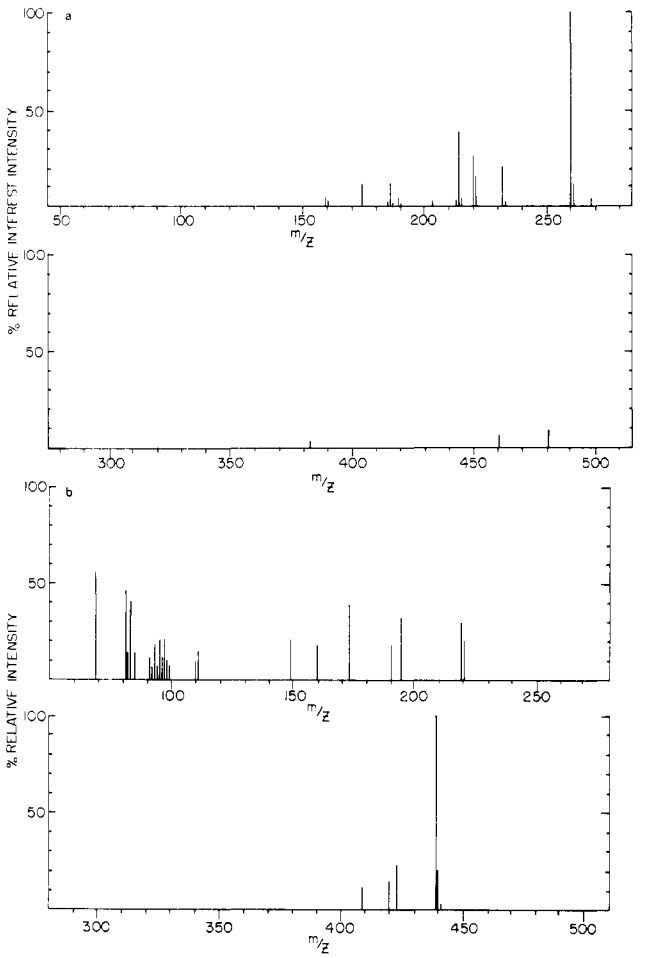

Figure 14. Mass spectra of (a) compound III and (b) compound VI.

yielded two major bands. The band showing the greatest migration (R_f 0.22) was purple and located immediately above an orange band (R_f 0.18). Extraction and crystallization of the purple band produced dark purple crystals which proved to be identical with IV by using TLC and IR spectroscopy. The orange band proved to be unreacted II. Many other less intense bands of lower R_f values were observed but not characterized. The reaction mixture of the $NaBH_4$ reduction, when subjected to TLC, showed many bands. However, only the most intense band was characterized. An orange band showing the greatest migration (R_f 0.77) was unreacted II.

Compound III (*N*-propyl-2,2′-azobis(α,α,α-trifluoro-6-nitro-*p*-toluidine)) (Figure 1) was isolated from the photolysis mixture of 20 g of trifluralin in quantities never exceeding 5 mg and representing less than 0.125% of the total photolysis products. It was purified as fine, purple needles (mp 182–183 °C).

The IR spectrum of III (Figure 4) indicated the presence of the following structures: aromatic C–H (3110 cm^{-1}), aliphatic C–H (1980–2880 cm^{-1}), aromatic nitro group (1530, 1310, 900 cm^{-1}), trifluoromethyl C–F (1120 cm^{-1}), primary amine N–H (3480, 3370 cm^{-1}), secondary amine N–H (3270 cm^{-1}). The secondary amine N–H stretch was shifted to a significantly lower frequency relative to the secondary amine absorptions in I and II. Hydrogen bonding in III could be responsible for the lower energy of the primary amine. The azo group is significantly less bulky than the azoxy group. As a result, the secondary amine group of III could more readily assume a configuration which favors hydrogen bonding than in the corresponding azoxy compound I. A difference in force constants for the N–H stretch in the azo compound as compared to the azoxy compound may also be of some importance. Due to the symmetry of the trans azo group, transitions producing IR absorption bands are forbidden. Unsymmetrically substituted azobenzenes give rise to an IR absorption band in the 1450–1500 cm^{-1} region (Colthup et al., 1975). A weak band at 1470 cm^{-1} may be assigned to the azo group, but due to the many aromatic and aliphatic bands in this region, the assignment should be accepted with caution.

The mass spectrum of III (Figure 14) has not been previously discussed in the literature. The molecular ion was evident at m/z 480.0980, which is consistent with the

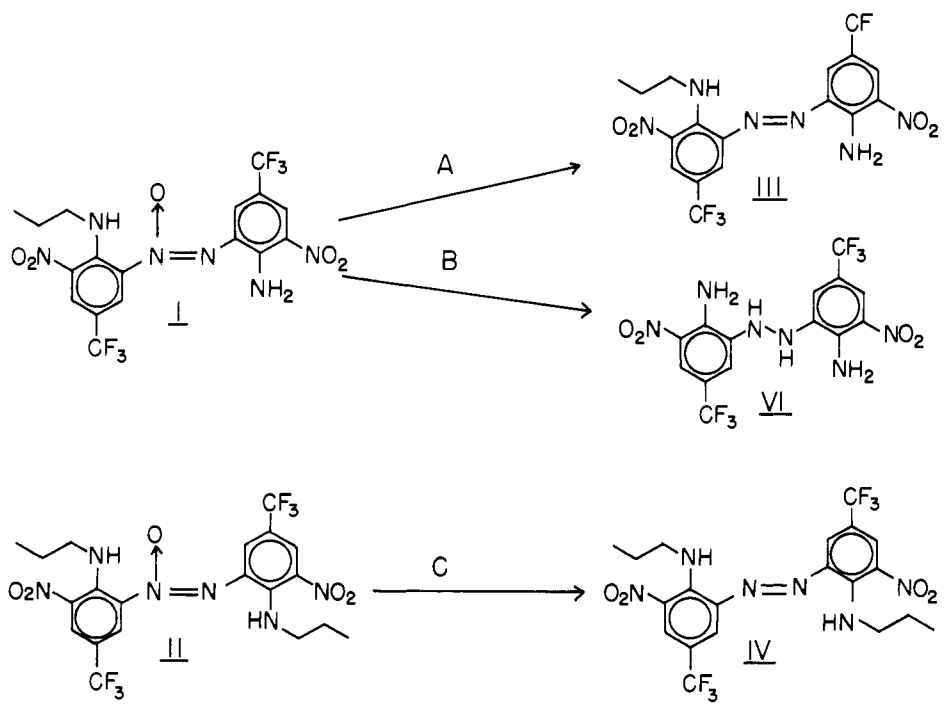

Figure 15. Chemical reductions of compounds I and II. Path A, $SnCl_2$–HCl or $NaBH_4$ or thermal decomposition. Path B, $NaBH_4$ reduction. Path C, $SnCl_2$–HCl reduction.

molecular formula $C_{17}H_{14}F_6N_6O_4$. Cleavage between the azo nitrogens and rearrangement of a hydrogen atom produced strong peaks at m/z 260.0642 and 220.0334. Lower mass fragments had m/z values consistent with loss of C_2H_5, NO_2, NH_2, and N. The pattern observed is exactly parallel to the fragmentation seen in the other azo and azoxy compounds discussed in this paper.

The NMR of III (Figure 11) showed only two well-separated, sharp peaks in the aromatic region of the spectrum (δ 8.60 and 7.95), both of equal area, indicating approximately two protons each. Comparison of the NMR of III with the NMR of the corresponding azoxy compound I suggested the singlet at δ 8.60 to be due to H_b and H_b' and the multiplet at δ 7.90 to be due to H_a and H_a'. In III, H_a and H_a' would be expected to have nearly the same chemical shift because of the symmetry of the azo group. The signals in the high field of the spectrum were consistent with a *N*-propyl group with coupling of the amine hydrogen to the α-methylene hydrogens. The α-methylene group showed somewhat more deshielding in III than in I. This difference in shift probably arose as a result of the stereochemistry of the molecule. Molecular models indicated that the α-methylene group is in a position to be further deshielded by the azoxy oxygen. (Two singlets at δ 1.30 and 1.60 were attributed to hydrocarbon impurities.)

Compound IV, purified from band E (2,2′-azobis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)) (Figure 1), was obtained in very small quantities never exceeding 2 mg, starting with 20 g of trifluralin, and represented less than 0.05% of the total photolysis products. IV was obtained in pure form as dark-purple, fine needles (mp 106.5–208 °C). The mass spectrum of IV was essentially the same as the mass spectrum of a compound reported by Golab and Occolowitz (1979), varying only in the relative intensities of some peaks. We found the base peak at m/z 43 instead of m/z 260, which was the second most intense peak. The base peak at m/z 43 may be due to C_3H_7. The fragmentation of IV, as for the other compounds discussed here, involved cleavage between the two azo nitrogens and rearrangement of a hydrogen atom, resulting in major peaks at m/z 260 and 262. Further cleavage, especially loss of C_2H_5, NO_2, and NH_2, explained the formation of other important peaks of lower mass.

The IR spectrum of IV (Figure 5) showed the following characteristic absorptions: secondary amine N–H (3300 cm^{-1}), aromatic C–H (3110 cm^{-1}), aliphatic C–H (2960–2880 cm^{-1}), aromatic NO_2 (1540, 1525, 1310, 900 cm^{-1}), trifluoromethyl group (1120 cm^{-1}). The 1500 cm^{-1} band of the nitro group appeared to be doubled. Again, the azo group did not produce a diagnostic absorption.

The low field of the NMR spectrum of IV (Figure 12) indicated the presence of two types of aromatic protons and one broad peak accordant with the amine hydrogens. From an examination of the environments of the four aromatic hydrogens, it is evident that two pairs are exactly equivalent: H_a and H_a' (δ 7.9) and H_b and H_b' (δ 8.6). The two *N*-propyl groups are also in identical environments and should produce identical signals. One set of signals characteristic of the *N*-propyl groups was seen: δ 3.75 (qd, J = 6 Hz, H_d'), 1.8 (s, J = 6 Hz, H_e and H_e'), 1.1 (t, J = 6 Hz, H_f and H_f'). The NMR spectrum of IV should vary from the NMR of III in only two respects. IV should show only one amine hydrogen peak, while III should show two amine hydrogen peaks. The integration of the signals should be different, with the aliphatic signals being twice as intense in IV than in III. Unfortunately, the small amounts of IV made the integration of its NMR impossible. The chemical shifts of the peaks in III and IV were expected to be essentially the same, which was the case. (Singlets at δ 1.30 and 1.60 appeared to be the same impurities mentioned above.)

Compound V from band E (2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*p*-toluidine)) (Figure 1a) was isolated from the photolysis mixture as crystals (mp 220–221 °C) which were dark-yellow plates. Less than 5 mg, representing approximately 0.125% of the photolysis products, was isolated, starting with 20 g of trifluralin.

The mass spectrum was essentially the same as that reported by Golab and Occolowitz (1979), except in our mass spectrum of this compound the molecular ion was also the base peak, m/z 454.0462. The second most intense peak was at m/z 173.0325, which was the base peak in their spectrum. Two other peaks at m/z 219.0259 and 235.0203 indicated that cleavage between the two azoxy nitrogens was the major path of fragmentation. Further fragmentation was exactly parallel to the fragmentation of compounds described above.

The IR spectrum of V (Figure 6) shows the presence of the following groups: primary amine (3480, 3360 cm^{-1}), aromatic C–H (3110 cm^{-1}), aromatic nitro group (1540, 1300, 910 cm^{-1}), trifluoromethyl group (1130 cm^{-1}), azoxy group (1460, 1315 cm^{-1}).

The NMR of V (Figure 13) (solvent acetone-d_6) was quite simple, exhibiting only peaks in the aromatic region of the spectrum. The pattern of the peaks was quite similar to those seen in the other azoxy compounds. Three sharp peaks representing the four aromatic hydrogens were observed. The peaks at δ 9.25 and 8.50 were split (J = 2 Hz). This may be attributed to either the presence of adjacent trifluoromethyl groups or meta coupling of meta hydrogens. Two broad peaks were observed at δ 8.25 and 7.90, which were assigned to the amine hydrogens. The NMR was consistent with the proposed structure and correlated well with the NMR spectra of the other azoxy compounds I and II.

Compound VI [1,2-bis(α,α,α-trifluoro-2-amino-3-nitro-*m*-tolyl)hydrazine] (Figure 15) was recrystallized twice from acetone–hexane as yellow crystals (mp 190 °C). The IR spectrum of VI (Figure 7) indicated the presence of the following functional groups: primary amine N–H (3500, 3380 cm^{-1}), aromatic C–H (3120 cm^{-1}), aromatic C–H (3120 cm^{-1}), aromatic NO_2 (1310, 1540, 910 cm^{-1}), and the trifluoromethyl C–F (1120 cm^{-1}). Hydrazobenzene shows absorptions near 3320 cm^{-1} (Pouchert, 1975) and *o*-tolylhydrazine shows no hydrazo N–H absorption bands in this region (Sadtler Research Laboratories, 1975). The IR spectrum of VI shows no absorption bands which indicate the presence of a hydrazo group.

The mass spectrum of VI (Figure 14b) showed an apparent molecular ion at m/z 440 and base peak at m/z 439, apparently due to loss of one hydrogen. Loss of either OH or NH_3 could be responsible for the peak at m/z 423 and loss of HF for the peak at m/z 420. The relatively intense peak at m/z 220 can be explained by fission between the two hydrazo nitrogens. The less intense peaks at m/z 236 and 205 were consistent with fragmentation between a hydrazo nitrogen and a ring carbon. The mass spectrum of a similar compound, 1,2-bis(α,α,α-trifluoro-2,6-dinitro-*p*-tolyl)hydrazine, produced a base peak consistent with cleavage between a hydrazo nitrogen and the ring carbon, with the loss of two hydrogens (Golab and Occolowitz, 1979). A very small peak (3% rel intensity) consistent with this fragmentation pattern was present in the mass spectrum of VI. Further fragmentation of VI was similar to that seen for the azobenzenes reported here. The three azoxybenzenes I, II, and V differ only in the number and

position of the *N*-propyl groups present in the molecule (Figure 1a), and the two azobenzenes III and IV were analogous to the azoxybenzenes I and II, respectively (Figure 1a). Photolysis of trifluralin in benzene solution yielded azobenzenes and azoxybenzenes in sufficient quantities to be a reasonably convenient route to their synthesis. Another compound, isolated in fairly large yield, was VII (Figure 1a). These compounds represented the major photolysis products out of some 30–40 compounds that were demonstrated by thin-layer chromatography.

Because of the large number of azo and azoxy compounds which have been shown to produce significant biological effects (Miyadera, 1975), the evaluation of the biological activity of these compounds is of some significance. Several azo and azoxy compounds such as 4-dimethylaminoazobenzene (DAB) are known to be carcinogenic. Although the mechanism of carcinogenicity of these compounds is not well understood, it is known that the biological activity is dependent upon the pattern of ring substitution; for instance, the 2-methyl derivative of DAB is noncarcinogenic (Chaveau et al., 1977). Quantities of three products I, II, and III were sufficient for mutagenesis testing by the method of Ames et al. (1975). None of the three compounds tested exhibited significant toxicity at concentrations from 0.001 to 1.0 mg/plate.

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Crystal and Molecular Structures of Organophosphorus Insecticides. 13. *S*-Isopropyl *O*-Methyl *O*-(3,5,6-Trichloro-2-pyridyl) Phosphoramidothioate and Dimethoate

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The crystal and molecular structures of *S*-isopropyl *O*-methyl *O*-(3,5,6-trichloro-2-pyridyl) phosphoramidothioate, $C_9H_{12}N_2O_2PSCl_3$, and dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoyl)methyl phosphorodithioate], $C_5H_{12}NO_3PS_2$, have been determined by three-dimensional X-ray analysis. The former compound crystallizes in the triclinic space group $P\bar{1}$ with a = 10.319 (5) Å, b = 10.730 (6) Å, c = 8.44 (4) Å, α = 99.01 (2)°, β = 114.02 (1)°, and γ = 62.64 (1)° with Z = 2, and the latter compound crystallizes in the monoclinic space group $P2_1$ with a = 6.574 (2) Å, b = 9.354 (2) Å, c = 9.885 (2) Å, and β = 107.4 (2)° with two molecules per unit cell. The structures were refined by a full-matrix, least-squares procedure to final residual indices of R = 0.058 and 0.068, respectively, using reflections with $F_o > 3\sigma(F_o)$. Atomic charge densities were calculated via CNDO/2 methods in order that positive charge center separations as well as geometric factors could be compared between these insecticides, acetylcholinesterase, and previous insecticides studied in this series.

In order to understand acetylchlolinesterase (AChE) inhibition by organophosphorus (OP) insecticides, it is desirable to know the three-dimensional structure of the active sites of this enzyme. Although the complexity of AChE makes direct elucidation of the structure difficult, accurate structural information on a large number of smaller molecules which interact strongly with the active sites of AChE should allow inferences to be made about the topography of the AChE molecule. A series of such structural studies has been carried out in this laboratory (Baughman and Jacobson, 1975, 1976, 1977, 1978a,b;

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