Amitrole Decomposition by Free Radical–Generating Systems and by Soils

J. R. Plimmer, P. C. Kearney, D. D. Kaufman, and F. S. Guardia

Amitrole (3-amino-1,2,4-triazole) decomposition was studied in free radical–generating systems: Fenton's reagent, ultraviolet irradiation, and riboflavin-sensitized photodecomposition. Fenton's reagent, which is a source of hydroxyl radicals, reacted with amitrole-5-^{14}C to give labeled CO_2, unlabeled urea, and unlabeled cyanamide. The other systems produced essentially similar results. In addition to products arising from ring cleavage, chromatographic evidence suggested that other products possibly arose by polymerization of amitrole radicals. Underlying similarities between the mode of breakdown of amitrole in free radical systems and in soils are discussed. On the basis of the reported free radical content of soils, radical processes may play some part in the decomposition of amitrole in soil.

The fate of a pesticide in soil depends on the properties of the chemical and the biological, chemical, and physical nature of its immediate environment. Much emphasis has been placed upon transformations of pesticides by soil microorganisms. However, whether a particular transformation has been mediated by biological or purely chemical reactions in soils has been difficult to delineate for many herbicides. Hydrolysis by acid or base may be cited as possible examples of chemical reactions which could occur in soils.

Steelink and Tollin (1967) state that the free radical population of soil organic matter is relatively high, and have suggested that free radical reactions may be of importance in soils.

Factors affecting the behavior of the herbicide amitrole (3-amino-1,2,4-triazole) in soils have been reviewed by several investigators (Ashton, 1963; Bondarenko, 1958; Ercegovich, 1957; Ercegovich and Frear, 1964; MacRae and Alexander, 1965; Massin, 1959; Sund, 1956). The major factors cited as being responsible for amitrole decomposition include soil microbiological activity, adsorption by soil constituents, and chemical reactions following complexing and adsorption. Evidence for the importance of chemical reactions in the decomposition of amitrole stems from studies conducted on soils sterilized by various methods (Ercegovich, 1957; Kaufman, 1967). A rapid disappearance of amitrole occurs in both nonsterile and chemically sterilized soils, but not in steam-sterilized soil.

Other products are said to arise from amitrole metabolism in plants, animals, and soils (Ashton, 1963; Carter and Naylor 1960; Fang *et al.*, 1964; Frederick and Gentile, 1961; Herrett and Bagley, 1964; Hilton *et al.*, 1963; Massin, 1959; Onley *et al.*, 1963). As many as 13 different compounds are derived from amitrole in plants (Herrett and Bagley, 1964), while a similar number have been reported in soils (Ashton, 1963). The identity of many of these compounds is uncertain, although two adducts, *N-s*-triazol-3-yl-glucosylamine and 3-amino-1*H*-1,2,4-triazole-1-alanine, have been isolated from plants and characterized (Frederick and Gentile, 1959; Massin, 1959). A hypothesis of amitrole action in plants based on its behavior towards free radical–generating systems has been proposed (Castelfranco and Brown, 1963; Castelfranco *et al.*, 1963). Experiments were reported on the photosensitization of amitrole in the presence of riboflavin; amitrole was also destroyed by the copper sulfate–ascorbic acid system. The former system is a source of photolytically produced free radicals, whereas the radicals originate from a chemical oxidation-reduction system in the latter case. The products of reaction were not isolated.

The possibility that free radical reactions in soils may be important in destroying amitrole has been pointed out recently (Kearney and Plimmer, 1967). The present study is based on the observation that the decomposition of amitrole in soil appears to be, in part, a chemical process, and that the heterocyclic ring of amitrole is readily susceptible to attack initiated by free radicals in contrast to its known stability towards a variety of common reagents. The present paper is concerned with the fate of amitrole in several systems in which free radicals are generated, and reports the identification of some of the products.

MATERIALS AND METHODS

Amitrole (98%) appeared homogeneous in all chromatographic systems used. 3-Hydroxy-1,2,4-triazole was synthesized by the method of Runti *et al.* (1959). Other reference compounds were purchased.

Infrared spectra were measured as KBr disks on a Perkin-Elmer Model 621 spectrophotometer. Mass spectra were determined on a Bendix Time of Flight Model No. 12 mass spectrometer.

Thin-layer chromatography was performed on plates 20 × 20 cm. or 2.5 × 7.6 cm. coated with silica gel G (E. Merck). 2-Propanol containing 3% concentrated ammonia solution, the most satisfactory solvent system for thin-layer chromatography, was used exclusively.

Paper chromatography was carried out on Whatman No. 1 paper with the following solvent systems: (a) 1-butanol–ethanol–water (4:1:1, v./v.); (b) 1-butanol–pyridine–water (1:1:1, v./v.). A modified nitroprusside spray reagent (Sund, 1956) was used for visualization of compounds examined.

$^{14}CO_2$ from labeled amitrole was measured by trapping in 2-methoxyethanol (7 ml.) and monoethanolamine (1 ml.). This solution was added to a scintillation solution (10 ml.)

Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

containing PPO (5 grams) and POPOP (0.15 grams) in toluene (1 liter). Radioactivity was measured in a Nuclear-Chicago Mark I liquid scintillation counter. All measurements were corrected for background and efficiency.

Irradiation of Amitrole in the Presence of Riboflavin. Amitrole and riboflavin were dissolved in water from which carbon dioxide had been displaced by boiling, followed by saturation with nitrogen. For initial studies, samples of mixtures of the two substances at a number of different concentrations were irradiated in thin quartz cuvettes with a Westinghouse sun lamp having peak emission at about 3100 A. The quantity of amitrole in the solution after a period of time was determined by the procedure of Burke and Storherr (1961), which is based on measurement of the absorbance of the colored product obtained by coupling *N*-(1-naphthyl)ethylenediamine dihydrochloride with amitrole following nitrous acid treatment.

Amitrole concentrations varying from 0.06 to 0.89 m*M* and riboflavin concentrations from 0.09 to 0.26m*M* were used. The quantity of $^{14}CO_2$ evolved, as a percentage of that originally present, was used as a second parameter to measure the extent of reaction. Reaction products were examined by thin-layer and paper chromatography.

Further photochemical studies were carried out with a Hanovia 450-watt, high-pressure, quartz mercury vapor amp (Hanovia Catalog No. 679-A-36) housed in a water-cooled double-walled quartz immersion well, which was immersed in the solution to be irradiated contained in a cell of 1-liter working volume. Nitrogen gas was bubbled through the solution during the period of irradiation.

For later studies on riboflavin-photosensitized oxidation, light of wavelengths less than 2800 A. was prevented from reaching the solution by a borosilicate glass absorption sleeve inside the quartz well. Subsequent studies on the photodecomposition of amitrole alone were carried out in the above apparatus without the use of an absorption sleeve.

Irradiation of Amitrole. A solution of amitrole (0.2 gram) in water (1 liter) was irradiated as described (no filter) for 6 hours. The combined solutions from three such preparations were evaporated in a rotary evaporator. The residue was dissolved in 2-propanol containing 3% concentrated aqueous ammonia, and put onto a silica gel column (E. Merck 60- to 80-mesh, 60 grams). Fractions of 1.5 ml. were collected automatically and examined by thin-layer chromatography. After 140 ml. of solvent had been collected, fractions containing a fast-moving substance which gave a purple color with the nitroprusside reagent were obtained. The identification of this compound is described later. A substance which appeared to be unchanged amitrole was next eluted from the column.

In a separate experiment, amitrole (0.1 gram) was irradiated in water (1 liter) for 6 hours (no filter). One microcurie of amitrole-5-^{14}C was also present in the solution.

The solution was evaporated, and the products were examined on thin-layer chromatography. The products were visualized by the modified nitroprusside spray and by contact with a photographic film (Figure 1).

Oxidation by Fenton's Reagent. Typical reaction conditions (Brown *et al.*, 1964) were as follows: A 0.2*M* solution of ferrous sulfate in 0.1*M* sulfuric acid (60 ml.) was added to a solution of radioactive amitrole in water (0.95 gram in 25 ml.) and 1% hydrogen peroxide (100 ml.) in 0.05*M* sulfuric acid was added to the mixture. After 15 minutes, a further quantity of 1% hydrogen peroxide (15 ml.) was added.

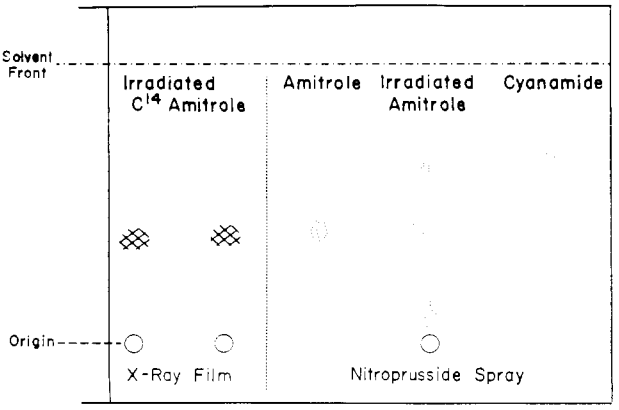


Figure 1. Thin-layer chromatogram showing the mobility of irradiated amitrole-5-C^{14}, unlabeled amitrole, and cyanamide

Labeled amitrole was detected on no-screen x-ray film. Amitrole and cyanamide were detected by the nitroprusside spray reaction

The $^{14}CO_2$ evolution was measured, and it was calculated that 46% of the total radioactivity was obtained as $^{14}CO_2$ after 5 hours.

In a separate experiment in which amitrole (10 mg.) was made to react with ferrous sulfate 0.2*M* (25 ml.) in 0.1*M* sulfuric acid and 1% hydrogen peroxide (50 ml.) for 2 hours, 80% of the radioactivity was obtained as $^{14}CO_2$. Products were examined by thin-layer chromatography.

The oxidation procedure was repeated to isolate products. The solution was evaporated to dryness in the rotary evaporator under reduced pressure. The dry material was extracted several times with hot ethanol. The ethanol was concentrated to give a sirup which was dissolved in water and shaken with Dowex-1 ion exchange resin (OH^-) to remove acid. The aqueous extract was evaporated and afforded a substance which crystallized, and could be recrystallized from ethyl acetate containing a little methanol. The identification of this compound is described later.

RESULTS

The following systems have been studied: (1) riboflavin-photosensitized decomposition of amitrole; (2) photochemical breakdown of amitrole by ultraviolet radiation (ca. 2200 A.); and (3) oxidation of amitrole by hydroxyl radicals (Fenton's reagent).

Labeled carbon dioxide was evolved in systems 1 and 3 from amitrole-5-^{14}C. A number of products were observed in every case and these were separated by thin-layer chromatography and visualized by means of the modified nitroprusside spray which appears effective for compounds containing $—CO.NH_2$ or $—C{=}NH.(NH_2)$ units.

Some of these products were not labeled with ^{14}C. One of the unlabeled products [$R_{amitrole}$ 1.3 in solvent system (b) and 1.4 in solvent system (a)], which gave a purple spot with the modified nitroprusside spray, was identified as

cyanamide. A small quantity of this material was obtained as a sirup by column chromatography.

The mass spectrum gave only limited information, as the interpretation was confused by peaks of high mass, presumably due to polymerization, but the most intense signals were at *m/e* 41 and 42. An aqueous solution of the unknown gave a yellow precipitate with ammoniacal silver nitrate. The unknown gave a yellow color on paper when sprayed with Ehrlich's reagent (Waldi, 1965) and a purple-brown color when sprayed with the modified nitroprusside spray. The reactions indicate the presence of an amino group, and the presence of a band in the region of 2250 $cm.^{-1}$ in the infrared indicates that a nitrile (C≡N) is also present.

A comparison of the infrared spectrum of authentic cyanamide and cochromatography on thin-layer and on paper confirmed the identity of the compound. Cyanamide was detected in all three systems studied and was isolated, accompanied by urea, from the oxidation of aminoguanidine by Fenton's reagent. Aminoguanidine and amitrole have certain structural features in common. Since aminoguanidine is a derivative of hydrazine, a strong reducing agent, it would not be expected to arise from amitrole under oxidizing conditions. The oxidation pathways of the two compounds might be expected to have features in common, since the oxidation in both cases probably involves the ultimate loss of the N—N fragment as nitrogen.

Aminoguanidine, semicarbazide, formamidine, formamide, 3-hydroxytriazole, urea, and cyanamide were used for chromatographic comparison with the products of amitrole breakdown; of these, only urea and cyanamide were positively identified. Cyanamide was present in all cases and urea was isolated from the reaction of amitrole with Fenton's reagent. Like cyanamide, the urea was unlabeled. Urea was obtained by ethanolic extraction of the residue resulting from evaporation of the reaction mixture from Fenton's reagent oxidation of amitrole. The compound crystallized after deionization on Dowex-1 (OH^-) resin and was recrystallized from ethyl acetate containing a little methanol. Its identity was established by cochromatography with authentic material, by comparison of infrared spectra (Figure 2) and by melting point (unknown m.p. 130° C.; urea has m.p. 132° C.).

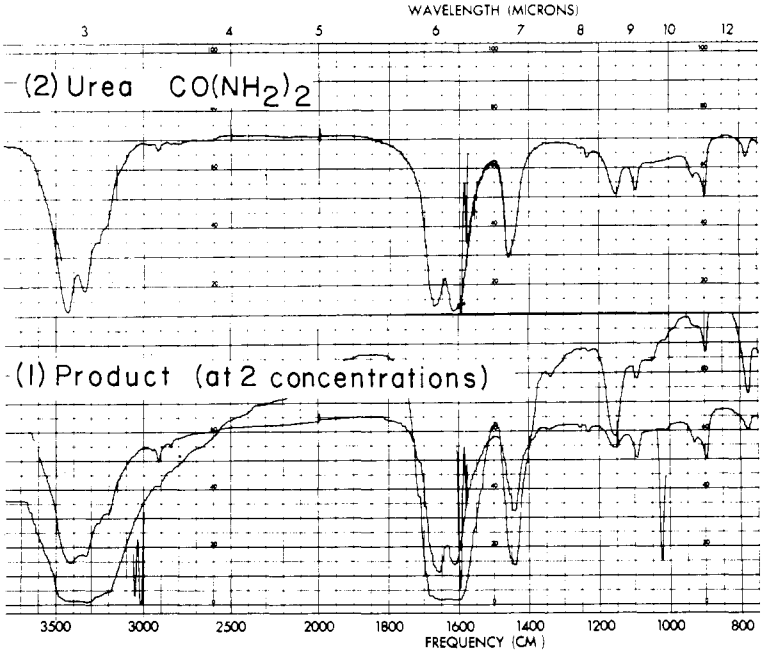


Figure 2. Infrared spectrum of urea and a product isolated from the reaction of amitrole with Fenton's reagent

The stoichiometry of these reactions has not been established but yields of $^{14}CO_2$ have been compared in all cases.

In the riboflavin-photosensitized decomposition of amitrole (oxygen excluded), when concentrations of 0.09 mmole per liter of riboflavin to 0.24 mmole per liter of amitrole were used, the concentration of amitrole was zero after 3 hours' irradiation when estimated by diazotization and coupling (Burke and Storherr, 1961). On the other hand, in the presence of excess riboflavin (0.26 mmole per liter), amitrole (0.06 mmole per liter) was oxidized and 10% of the label was evolved as $^{14}CO_2$ after 8 hours of irradiation. When amitrole (0.89 mmole per liter) was present in excess over riboflavin (0.13 mmole per liter) only 0.1% of the label was evolved as $^{14}CO_2$ after the same period. In the experiments using Fenton's reagent as oxidant, up to 80% of the label was recovered as $^{14}CO_2$.

DISCUSSION

Reactions leading to the breakdown of amitrole in the systems studied are complex but possess underlying similarities. It has not been possible to establish the quantitative features of the reactions so far, but in the case of Fenton's reagent, C-5 of amitrole apparently is rapidly and almost quantitatively oxidized to CO_2 by an excess of reagent. The photosensitized process seems to require a large excess of riboflavin to effect oxidation to CO_2, but destruction of amitrole, as estimated by the diazo reaction, is quickly brought about in a 3 to 1 molar ratio of amitrole to riboflavin. The presence of unlabeled cyanamide in the products of photosensitized decomposition, as well as in the products from amitrole irradiated by short wavelength ultraviolet radiation, leads to the inference that ring opening has occurred in both cases.

The course of the reactions appears to bear some similarity to that following attack by hydroxyl radicals (Fenton's reagent). The hydroxyl radical apparently attacks the ring at carbon atom 5 and ring cleavage occurs. A large percentage of the radioactivity may be recovered as $^{14}CO_2$. Compounds isolated, which originate from the remaining portion of the ring, are urea ($NH_2.CONH_2$) and cyanamide ($NH_2—C≡N$). These compounds are unlabeled. It has not been possible to isolate other intermediate products, although the occurrence of other unstable entities may be envisaged. Aminoguanidine ($NH_2.C=NH.NHNH_2$), which is related to amitrole, also gives rise to cyanamide and urea when oxidized by Fenton's reagent.

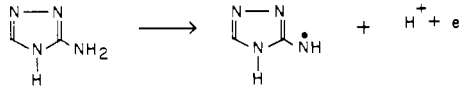
Cyanamide is a reactive compound; it may give rise to urea during the process of isolation. It may also form a dimer. Cyanamide is stable only in aqueous solutions which are weakly acid (Sidgwick, 1942).

The complete oxidation of amitrole to CO_2 is not easily achieved in the laboratory, since this compound is stable to many common oxidizing agents. In view of these observations, the behavior of amitrole was studied under a variety

of conditions. Oxidative breakdown was brought about most readily under conditions which favor free radical reactions.

In view of recent studies in which the free radical content of soil has been measured (Steelink and Tollin, 1967), it is important to consider such a mechanistic pathway, mediated by free radicals originating in soil, as a breakdown route for amitrole or, indeed, other pesticides applied to soil. A number of investigators have studied the free radical content of soil organic matter. Solid samples of humic acid are reported to have spin concentrations of the order of 10^{18} spins per gram measured by electron paramagnetic resonance spectrometry (Steelink and Tollin, 1967). Signals due to unpaired spins have been obtained from many soil components. The importance and reactivity of these free radicals are unknown, but Friedlander *et al.* (1963) have suggested that they may serve as scavengers for the halogenated pesticides.

While the oxidative processes described lead to the fragmentation of amitrole with the production of CO_2, urea, and cyanamide, the intact molecule may form a radical by loss of a proton and an electron (Castelfranco and Brown, 1963).



Such a radical could polymerize; and although no direct evidence for the presence of compounds of higher molecular weight than amitrole has been obtained, a number of labeled compounds which possess low chromatographic mobility have been detected. In addition, the photosensitized decomposition of amitrole by riboflavin results in rapid loss of ability to couple after attempted diazotization. The loss of carbon dioxide by oxidation is a much slower reaction that occurs in relatively low yield. Therefore, although breakdown of the amitrole ring occurs in the systems studied, polymerization may also take place. The proposed reactions of amitrole in a free radical system are summarized in Figure 3.

As noted previously, failure to detect any unlabeled products from breakdown of ^{14}C-5–labeled amitrole in soils is not surprising (Kaufman, 1967; Kaufman *et al.*, 1967).

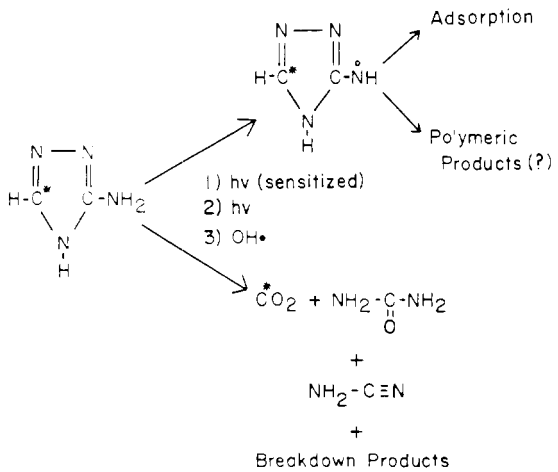


Figure 3. Proposed reactions of amitrole resulting from irradiation or oxidation with Fenton's reagent

If urea and cyanamide were produced in nonsterile soils, these compounds would rapidly be utilized by the soil microflora. If, in addition, the amitrole radical is formed, it could polymerize or react with various substrates. Such a process may explain the strong irreversible binding of amitrole to soil particles.

Work has been carried out in these laboratories by Kaufman (1967) on the fate of amitrole in soils. The rate of evolution of $^{14}CO_2$ from ^{14}C-labeled amitrole as a function of a number of soil parameters was studied. The experiments were designed to show the role of microorganisms in this process, but the observations led to the conclusion that the breakdown of amitrole is affected to a significant extent by nonbiological processes.

ACKNOWLEDGMENT

Amitrole (98%) was generously supplied by the American Cyanamid Co., Agricultural Division, Princeton, N.J. Amitrole-5-^{14}C was generously supplied by Amchem Products, Inc., Ambler, Pa.

LITERATURE CITED

Ashton, F. M., *Weeds* **11,** 167 (1963).
Bondarenko, D. D., *Proc. New England Weed Control Conf.* **15,** 5 (1958).
Brown, R. F., Jamison, S. E., Pandit, U. K., Pinkus, J., White, G. R., Brandlin, J., *Org. Chem.* **29,** 146 (1964).
Burke, J., Storherr, R. W., *J. Assoc. Offic. Agr. Chemists* **44,** 196 (1961).
Carter, M. C., Naylor, A. W., *Botan. Gaz.* **122,** 138 (1960).
Castelfranco, P., Brown, M. S., *Weeds* **11,** 116 (1963).
Castelfranco, P., Oppenheim, A., Yamaguchi, S., *Weeds* **11,** 111 (1963).
Ercegovich, C. D., Ph.D. thesis, Pennsylvania State University, University Park, Pa., 1957.
Ercegovich, C. D., Frear, D. E. H., J. AGR. FOOD CHEM. **12,** 26 (1964).
Fang, S. C., George, M., Yu, Te Chang, J. AGR. FOOD CHEM. **12,** 219 (1964).
Frederick, J. F., Gentile, A. C., *Arch. Biochem. Biophys.* **92,** 356 (1961).
Frederick, J. F., Gentile, A. C., *Physiol. Plantarum* **12,** 862 (1959).
Friedlander, H. Z., Saldick, J., Frink, C. R., *Nature* **199,** 62 (1963).
Herrett, R. A., Bagley, W. P., J. AGR. FOOD CHEM. **12,** 17 (1964).
Hilton, J. L., Jansen, L. L., Hull, H. M., *Ann. Rev. Plant Physiol.* **14,** 353–384 (1963).
Kaufman, D. D., Weed Society of America (Abstr.), p. 78, 7th Meeting, Washington, D.C., 1967.
Kaufman, D. D., Plimmer, J. R., Kearney, P. C., Blake, J., Guardia, F. S., *Weeds* **15,** in press.
Kearney, P. C., Plimmer, J. R., Weed Society of America (Abstr.) p. 76, 7th Meeting, Washington, D.C., 1967.
MacRae, I. C., Alexander, M., J. AGR. FOOD CHEM. **13,** 72 (1965).
Massin, P., *Biochem. Biophys. Acta* **36,** 548 (1959).
Onley, J. H., Storherr, R. W., Culver, A. J., Jr., *J. Assoc. Offic. Agr. Chemists* **46,** 996 (1963).
Runti, C., Sindellari, L., Nisi, C., *Ann. Chim.* **49,** 1649–67 (1959).
Sidgwick, N. V., "Organic Chemistry of Nitrogen," p. 329, Oxford University Press, Oxford, 1942.
Steelink, C., Tollin, G., "Soil Biochemistry," pp. 147–69, A. D. McLaren, and G. H. Peterson, Eds., Marcel Dekker, New York, 1967.
Sund, K. A., J. AGR. FOOD CHEM. **4,** 57 (1956).
Waldi, D., "Thin-Layer Chromatography," p. 490, E. Stahl, Ed., Academic Press, New York, 1965.

Received for review June 15, 1967. Accepted September 12, 1967. Mass spectral studies were performed on a contractual basis by Melpar, Inc., Falls Church, Va. Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.