Metabolism of [2-^{14}C]Terbacil in Alfalfa

Robert C. Rhodes

Alfalfa, treated with [2-^{14}C]terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) in the dormant stage at a rate of 1 lb of active ingredient/acre, contained average radiochemical residues of 2.2 and 0.4 ppm (calculated as terbacil) at the first and second harvests, 6 and 8 months after treatment, respectively. TLC analyses of extracts from the treated alfalfa showed a total of six ^{14}C-labeled areas. Three compounds were identified by mass spectral analyses; terbacil (12.5% of the ^{14}C in the extract), 3-*tert*-butyl-5-chloro-6-hydroxymethyluracil (metabolite A, 11.9%) and 6-chloro-2,3-dihydro-7-(hydroxymethyl)-3,3-dimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (metabolite B, 41.2%). The structures of metabolites C and D, 18.3 and 5.6%, respectively, were assigned on the basis of their R_f values on the TLC plates. The remainder of the ^{14}C activity (10.5%) was material at the origin of the TLC plate. No 5-chlorouracil was detected (<0.05%).

Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) is a selective herbicide for control of many annual and some perennial weeds in crops such as sugarcane, apples, peaches, citrus, and peppermint. It is marketed as a wettable powder containing 80% active ingredient under the tradename Sinbar terbacil weed killer by E. I. du Pont de Nemours & Co., Inc. The metabolism of terbacil in dogs (Rhodes et al., 1969), soil (Gardiner et al., 1969; Zimdahl et al., 1970; and Wolf and Martin, 1974) and orange seedlings (Jordan et al., 1975) have been reported. Recently Holt and Pease (1977) have reported a residue method for the determination of terbacil and its major metabolites.

EXPERIMENTAL SECTION

Radiolabeled Material. [2-^{14}C]Terbacil was synthesized according to the procedure described by Gardiner et al. (1969). The sp act. was 4.14 μCi/mg and the radiochemical purity was 97.5%. The reference standards of terbacil and metabolites were obtained from Biochemicals Department, Agrichemicals Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Crop Treatment. Small individual soil plots at Newark, Del., were isolated from the surrounding soil by inserting stainless steel boxes (1 × 1 × 1 ft open at top and bottom) into the ground, leaving about 1 in. of rim protruding above ground level to minimize run-off. The soil type was Keypott silt loam: pH 5.4; cation-exchange capacity, 8.9 me/100 g; organic matter, 2.3%; clay, 21%; silt, 70%; and sand, 9%.

Each isolated area contained two or three alfalfa plants. During January, when the alfalfa was in the dormant stage, four of the isolated areas were each treated with [2-^{14}C]terbacil at a rate of 1 lb of active ingredient/acre (10.4 mg, 43.1 μCi) by pipetting [2-^{14}C]terbacil evenly on the soil surface in 25 mL of water. The remaining two areas were used as untreated controls. The plants in each of these plots were harvested twice the following summer (June and August), 6 and 8 months after treatment by cutting at ground level.

Analysis. The plants from each plot and 150 mL of water were placed in a blender and homogenized at high speed for 5 min and the mixture freeze-dried. Aliquots of each sample (equivalent to 0.20 to 0.50 g, fresh weight) were analyzed for total ^{14}C residues by the combustion liquid scintillation counting method described by Smith et al. (1964).

Biochemicals Department, Research Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19898.

Alfalfa (93.0 g from plot 3, first harvest) was macerated with 100 mL of methanol in a blender for 5 min. The plant tissue and methanol were quantitatively transferred to a Soxhlet extractor and extracted with methanol for 64 h. The volume of the solution was reduced to ca. 25 mL (water) in a hood at room temperature.

The extracted plant tissue was placed in a blender and extracted an additional two times with 150 mL of 5% potassium hydroxide (Pease, 1968). The basic extracts were combined, adjusted to pH 7 and combined with the aqueous phase above. The resulting solution was extracted with ethyl acetate for 72 h in a continuous liquid–liquid extractor. The ethyl acetate extract was reduced to about 4 mL in a hood, quantitatively transferred to a 5-mL volumetric flask and made to volume with ethyl acetate. Aliquots of the aqueous phase and ethyl acetate extract were counted in a liquid scintillation spectrometer (Nuclear Chicago Model 6801) to determine the total ^{14}C in each phase. The extracted tissue was air dried and aliquots were analyzed for total unextracted radioactivity by combustion–liquid scintillation counting.

A 100-μL aliquot of the organic extract was applied to a thin-layer plate of kieselgel (250 μm thickness) as a streak and terbacil and metabolite A reference spots were applied next to the streak. The plate was developed to 15 cm in a mixture of hexane–ethyl acetate–methanol (10:10:1 v/v/v). The TLC plate was then placed next to Ansco nonscreen x-ray film for 3 weeks to determine the location of radiolabeled compounds. Areas of adsorbent, corresponding to radioactive materials, were removed from the plate and added to scintillation vials. Scintillation solution (15 mL) was added, and the vials were stored in the dark for 3 days, then counted to determine the total activity in each sample. A 100-μL aliquot of the organic extract was analyzed specifically for 5-chlorouracil according to the procedure reported by Rhodes et al. (1969) and Gardiner et al. (1969). The remaining organic extract (4.8 mL) was separated by TLC as above. The kieselgel corresponding to each radioactive material was removed from the plate and the radioactivity washed from the gel with 100 mL of methanol. Each radiolabeled compound was purified by several additional TLC steps. Mass spectra were obtained on terbacil and two isolated degradation products using a Bendix Model 12-107 time-of-flight mass spectrometer (Reiser, 1969).

RESULTS AND DISCUSSION

The results of analyses for total ^{14}C, listed in Table I, show an average total radiochemical residue equivalent to 2.2 ppm (calculated as terbacil) in the alfalfa that was harvested 6 months after treatment and 0.4 ppm 8 months after treatment.

Table I. Residue Analyses of [2-^{14}C]Terbacil Treated Alfalfa

Plot	Treatment rate, lb of AI/acre	^{14}C equivalent to ppm of terbacil[a]	
		1st harvest[b]	2nd harvest[c]
1	Control	<0.02	0.02
2	Control	<0.02	0.02
3	1	3.5	0.3
4	1	1.9	0.5
5	1	1.1	0.5
6	1	3.1	0.4
	Weighted average	2.2	0.4

[a] Based on fresh weight. [b] Six months after treatment. [c] Eight months after treatment.

Table II. Percent of Terbacil and Metabolites in Alfalfa Extracts

Compound	% of extract	ppm
Terbacil	12.5	0.44
Metabolite A	11.9	0.44
Metabolite B	41.2	1.53
Metabolite C	18.3	0.63
Metabolite D	5.6	0.18
At origin	10.5	0.37

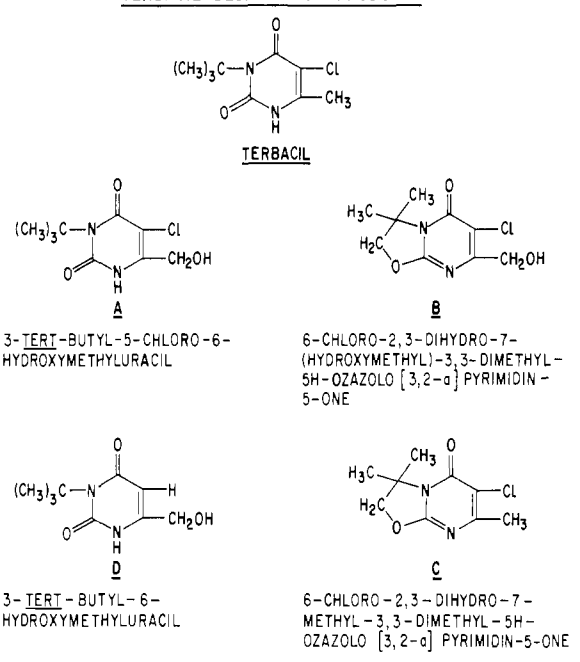

Figure 1. Terbacil degradation products.

The major degradation products of [2-^{14}C]terbacil in alfalfa, shown in Figure 1 and listed in Table II, are 3-*tert*-butyl-5-chloro-6-hydroxymethyluracil (A), 6-chloro-2,3-dihydro-7-(hydroxymethyl)-3,3-dimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (B), and 6-chloro-2,3-dihydro-7-methyl-3,3-dimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (C). TLC separations of the 6-month plant extract showed that 12.5% of the extracted radioactivity was intact terbacil and that 11.9% was A, 41.2% B, 18.3% C, and 5.6% D. Specific analyses showed that no 5-chlorouracil (<0.05%) was present as a terbacil metabolite. The extraction procedure removed 86.3% of the ^{14}C in the organic phase, 10.2% in the aqueous fraction, and 3.6% was unextracted. Previous studies (Gardiner et al., 1969; Pease, 1968) showed that terbacil is not altered by the extraction procedure used in this work. Gardiner et al. (1969) showed that greater than 90% of the radioactive residue in soil treated with [^{14}C]terbacil was unchanged parent compound

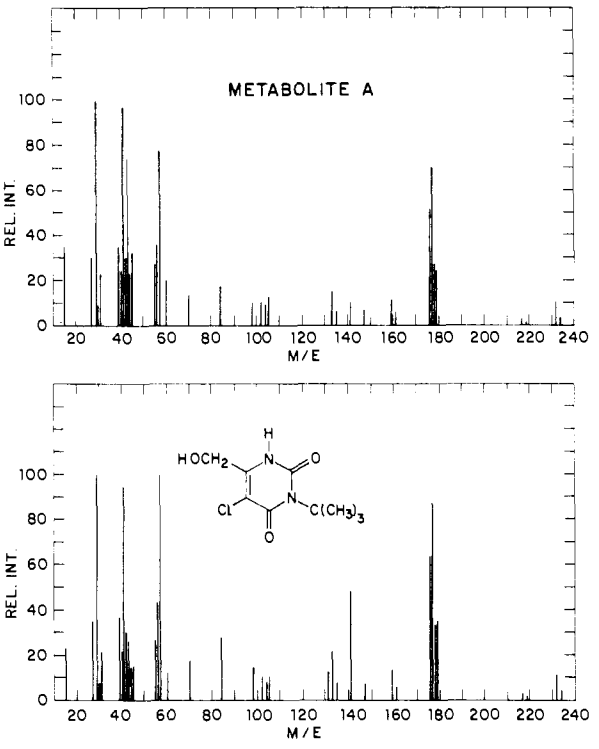

Figure 2. Upper, mass spectrum of metabolite A, isolated from treated alfalfa; lower, mass spectrum of 3-*tert*-butyl-5-chloro-6-hydroxymethyluracil.

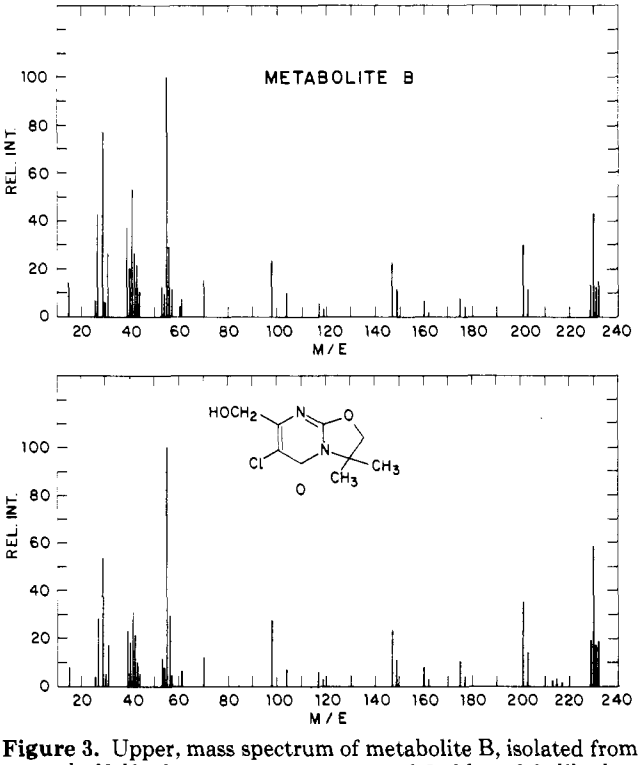

Figure 3. Upper, mass spectrum of metabolite B, isolated from treated alfalfa; lower, mass spectrum of 6-chloro-2,3-dihydro-7-(hydroxymethyl)-3,3-dimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one.

for exposure periods up to 1 year. Therefore, it is probable that the isolated degradation products were formed in the plant.

The structures of terbacil and compounds A and B were confirmed by comparison of the mass spectra of the isolated materials with the spectra of reference standards (Figures 2 and 3). Metabolites C and D were identified by comparison of their TLC R_f values with R_f values of

reference standards. All the degradation products listed in Table II have been reported as metabolites of terbacil in dog urine (Rhodes et al., 1969). Jordan et al. (1975) have reported that metabolite A is the major metabolite in orange seedlings, cultured in aqueous solutions of [2-^{14}C]terbacil and that 5-chlorouracil was not detected as a metabolite of terbacil.

ACKNOWLEDGMENT

The author gratefully acknowledges R. W. Reiser for obtaining and interpreting the mass spectra.

LITERATURE CITED

Gardiner, J. A., Rhodes, R. C., Adams, J. B., Soboczenski, E. J., *J. Agric. Food Chem.* **17**, 980 (1969).
Holt, R. F., Pease, H. L., *J. Agric. Food Chem.* **25**, 373 (1977).
Jordon, L. S., Zurqiyah, A. A., Clerx, W. A., Leasch, J. G., *Arch. Environ. Contam. Toxicol.* **3**, 268 (1975).
Pease, H. L., *J. Agric. Food Chem.* **16**, 54 (1968).
Reiser, R. W., *Org. Mass Spectrom.* **2**, 467 (1969).
Rhodes, R. C., Reiser, R. W., Gardiner, J. A., Sherman, H., *J. Agric. Food Chem.* **17**, 974 (1969).
Smith, G. N., Ludwig, P. D., Wright, K. C., Bauriedel, W. R., *J. Agric. Food Chem.* **12**, 172 (1964).
Wolf, D. C., Martin, J. P., *Soil Sci. Soc. Am. Proc.* **38**, 921 (1974).
Zimdahl, R. L., Freed, V. H., Montgomery, M. L., Furtick, W. R., *Weed Res.* **10**, 18 (1970).

Received for review February 14, 1977. Accepted May 10, 1977.

Photolysis of 3-(4-Chlorophenyl)-1,1-dimethylurea in Dilute Aqueous Solution

Fred S. Tanaka, Ronald G. Wien, and Richard G. Zaylskie

Saturated aqueous solutions of 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) of approximately 200 ppm concentration were photolyzed. Irradiations were performed with either a 450 W Hanovia high-pressure mercury arc or a Rayonet photochemical reactor fitted with sunlight lamps. Light from both systems was filtered through Pyrex glass to more closely resemble sunlight by elimination of the shorter wavelength bands. Eleven products were identified from the photoreaction. Ring hydroxylation, methyl oxidation, N-demethylation, dechlorination, and dimerization were the main processes that occurred. The same products were obtained with both light sources, but the distributions of product yields were slightly different. Yields were measured at various exposure levels for principal photoproducts, and the quantum efficiency for monuron loss (Φ = 0.1) and photoproduct formation were estimated.

The first indication that substituted phenylurea herbicides were affected by ultraviolet radiation was the observed decrease in the herbicidal activity of these compounds with exposure to sunlight (Hill et al., 1955; Weldon and Timmons, 1961; Comes and Timmons, 1965). Since this initial observation, interest has developed in the photolysis of substituted phenylureas with particular emphasis on the photodegradation of 3-(4-chlorophenyl)-1,1-dimethylurea (monuron, cf. structure I, Table I). Preliminary studies were carried out by Jordan and coworkers (1964) who investigated the change in absorption spectra of monuron with increased dosage of ultraviolet light. A more extensive study was performed by Crosby and Tang (1969) in which saturated aqueous solutions of monuron were irradiated by natural sunlight and by blacklight fluorescence. Four photoproducts (II, III, IV, VII) were clearly identified, and the partial characterization of four additional photoproducts was given. Rosen et al. (1969) examined the photolysis of monuron in aqueous solution by sunlight, and the hydroxylated photoproduct (V), a previously unobserved product, was identified. Although other photoproducts were obtained in this study, identification of these products was not attempted because of the extensive work by Crosby and Tang (1969). Mazzocchi and Rao (1972) examined the photochemistry of methanolic solutions of monuron under nonoxygenated conditions employing a low-pressure mercury lamp (254 nm). A photorearrangement similar to the photo-Fries reaction of aryl esters and the analogous rearrangement of anilides was observed. The identified rearrangement products were the 2-amino- and 4-amino-*N*,*N*-dimethylbenzamide.

We extended the study on the photolysis of monuron to include the identification of additional photoproducts and the estimation of product yields. Although the conditions employed were not strictly those observed in the environment, the results may give some indication as to what might be expected under environmental conditions since the studies were performed in aqueous oxygenated solutions.

EXPERIMENTAL SECTION

Materials. Monuron was prepared by reaction of a chloroform solution of 4-chlorophenylisocyanate (K and K) with dimethylamine (Eastman). The product was initially recrystallized several times from chloroform and finally recrystallized from isopropanol until a homogeneous product with a melting point of 172–173 °C (uncorrected) was afforded. Spectral properties that verify the identity of the synthetic product are given in the Identification of Photoproducts section. Previously synthesized [*ring*-^{14}C]monuron (Tanaka, 1970) was repurified before use by the thin-layer chromatography (TLC).

Equipment. Spectrometric measurements for actinometry were taken on a Beckman DB spectrophotometer. Infrared (IR) spectra were recorded on a Perkin-Elmer 337 spectrophotometer equipped with beam condenser. The samples were analyzed in 1.5 mm micro KBr pellets. Mass spectra were obtained on a Varian CH-5DF spectrometer at 70 eV. The samples were introduced by means of a temperature-programmed solid sample probe. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian A-60A spectrometer equipped with Fourier transform (Digilab) capability. Tetramethylsilane was employed as the internal standard. Preparative TLC for

U.S. Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58102.