Degradation of Chlorbromuron and Related Compounds by the Fungus *Rhizoctonia solani*1

MARTIN WEINBERGER AND JEAN-MARC BOLLAG

Laboratory of Soil Microbiology, Department of Agronomy, The Pennsylvania State University, University Park, Pennsylvania 16802

Received for publication 11 July 1972

The ability of the soil fungus *Rhizoctonia solani* to degrade phenyl-substituted urea herbicides was investigated. The fungus was able to transform chlorbromuron [3-(3-chloro-4-bromophenyl)-1-methyl-1-methoxyurea] to the demethylated product [3-(3-chloro-4-bromophenyl)-1-methoxyurea], which was isolated and identified. Evidence was obtained that further degradation of chlorbromuron occurred. Several other phenylurea compounds (chloroxuron, diuron, fenuron, fluometuron, linuron, metobromuron, neburon, and siduron) were also metabolized by the fungus, indicating that *R. solani* may possess a generalized ability to attack this group of herbicides.

Biochemical activity of the soil microflora is assumed to be a major factor for removal of phenylurea herbicides from soil (4, 6), and several reports have been made concerning the microbial degradation of these compounds (2, 3, 7, 8). However, there is a noticeable lack of detailed microbial and biochemical studies on transformations of the phenylurea herbicides and especially on the participation of fungi, which constitute the largest portion of the total microbial protoplasm in most cultivated soils (1).

Hill and McGahen (3) reported first on the involvement of fungi, *Penicillium* and *Aspergillus* spp., in the transformation of monuron [3-(p-chlorophenyl)-1,1-dimethyurea]; they claimed that it can be used as a carbon source in an agar medium. The first specific data on fungi were presented by Tweedy et al. (7), who found that *Talaromyces wortmanii* and *Fusarium oxysporum* metabolize metobromuron [3-(3-bromophenyl)-1-methoxy-1-methylurea]. They isolated 1-(3-bromophenyl)-3-methoxyurea and 1-(p-bromophenyl)-3-methylurea which indicated a dealkylation and a dealkoxylation reaction. They were also able to identify *p*-bromophenurea as an intermediate. From the finding of *p*-bromoacetanilide, they concluded that an acetylation reaction is involved in the detoxication of metobromuron.

The present study was designed to investigate the ability of a selected soil fungus to degrade chlorbromuron [3-(3-chloro-4-bromophenyl)-1-methoxy-1-methylurea] and other phenylurea herbicides.

**MATERIALS AND METHODS**

Fungal cultures were grown in mycological broth (Difco) supplemented by the addition of 1 g of MgSO4·7H2O per liter and a trace element solution containing: Na2B4O7·10H2O, 400 µg; CuSO4·5H2O, 400 µg; FeSO4·7H2O, 800 µg; MnSO4·2H2O, 800 µg; and ZnSO4·7H2O, 8 mg per liter.

Chlorbromuron and other herbicidal compounds were dissolved in absolute ethanol, sterilized by membrane filtration (0.22-µm pore size Millipore filter), and added to the growth media. The ethanol concentration in the final growth medium was 1%.

All cultures were grown in 250-ml flasks containing 60 ml of media and were incubated at 30°C on a rotary incubator shaker (250 oscillations/min). At least two replicates were included in each treatment, and each experiment was repeated two or three times. Larger cultures from which chlorbromuron breakdown products were isolated consisted of 750 ml of media in a 2-liter flask.

The following chemicals were obtained from CIBA Agrochemical Co. (Vero Beach, Florida): chlorbromuron, 3-(3-chloro-4-bromophenyl)-1-methyurea, 3-(3-chloro-4-bromophenyl)-1-methoxyurea, 3-(3-chloro-4-bromophenyl) urea, 3-chloro-4-bromoaniline, and 14C-carbonyl-labeled chlorbromuron (specific activity 6.5 µCi/mg). Technical grade linuron (96.5%) [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], neburon (98.8%) [3-(3,4-dichlorophenyl)-1-butyl-1-methylurea], siduron (97.3%) [3-phenyl-1-(2-methylcyclohexyl) urea], diuron (97.5%) [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and fenuron (99.5%) [3-phenyl-1,1-dimethylurea] were provided by E. I. Du Pont de Nemours Co., Inc. (Wilmington, Delaware).
DEGRADATION OF CHLORBROMURON BY RHIZOCTONIA

Vol. 24, 1972

Vegetable grade chloroxuron [2-(4-(p-chlorophenoxy)-phenyl)-1,1-dimethylurea], fluometuron [3-(m-trifluoromethylphenyl)-1,1-dimethylurea], and metobromuron were supplied by CIBA Agrochemical Co.

Radioactivity was determined with a Nuclear-Chicago Mark I liquid scintillation counter. Aqueous samples were measured in a Bray solution composed of 60 g of naphthalene, 100 ml of methanol, 20 ml of ethylene glycol, and 8 g of Omnifluor 98% PFO (2,5-diphenyloxazole), 2% Bis-MSB [p-bis-(O-methylstyril)benzene]; New York Nuclear Corp., Boston, Mass.) in 1 liter of dioxane. Radioactive compounds from thin-layer chromatograms were counted by scraping the entire spot into a scintillation solution containing 4 g of Omnifluor and 40 g of Cab-O-Sil (Thixotropic suspension powder, Cabot Corp., Boston, Mass.) per liter of toluene.

Precoated thin-layer plates (silica gel F-254) with fluorescent indicator were purchased from Brinkman Instruments, Inc. (Westbury, N.Y.). Routine thin-layer analyses and preparative separations were carried out with layer thicknesses of 0.25 and 0.50 mm, respectively. Two solvent systems were employed in all analyses to ascertain the validity of the thin-layer data: ether-hexane (4:1, v/v) and methylene chloride-acetonitrile (4:1, v/v).

All compounds except 3-chloro-4-bromoaniline were made visible with ultraviolet (UV) light. The aniline was detected by spraying the plate with an aromatic amine specific reagent composed of 1 g of dimethylamino benzaldehyde dissolved in 180 ml of 1-butanol, 30 ml of ethanol, and 3 ml of HCl.

For routine thin-layer analyses, 5 ml of filtered media was extracted twice with an equal volume of ether. The extracts were then combined, concentrated fifty times by heating in a water bath at 40°C, and spotted on the thin-layer plates.

Breakdown products of chlorobromuron were isolated by extracting the growth media twice with an equal volume of ether. The ether extract was then evaporated to dryness with a flash evaporator at room temperature. The resultant residue was dissolved in 2 ml of ether and analyzed on a preparative thin-layer plate. The compound being isolated was removed from the plate and purified two more times by thin-layer chromatography (TLC).

Gas chromatographic analyses were performed with a Packard gas chromatograph (model 7424) equipped with an electron capture detector. A 16-inch, 1/4-inch outer diameter, 3/8-inch inner diameter glass column packed with 1.5% XE-60 silicone gum on 80-100 mesh Gas Chrom Q (Supelco, Inc.) as described by Katz and Strusz (5) was used. The carrier gas (nitrogen) flow rate was 40 ml/min. Injector and detector temperatures were 225°C and 300°C, respectively. A column temperature of 170°C was routinely used, except that 190°C was utilized for determination of 3-(3-chloro-4-bromophenyl)-1-methylurea and 100°C was used for analysis of 3-(3-chloro-4-bromophenyl) urea and 3-chloro-4-bromoaniline.

Growth media samples were prepared for gas chromatographic examination by extracting twice with equal volumes of ether. The extracts were then combined and adjusted to give a theoretical concentration of 5 μg/ml.

Mass spectra were taken with a model 902 mass spectrometer (Associated Electrical Industries, Ltd., England) by using a direct probe technique; the samples were subjected to an ionization potential of 70 eV.

RESULTS

After screening a number of soil fungi for their ability to attack chlorobromuron, it was found that Rhizoctonia solani was most active in transforming the herbicide. When the fungus was grown in a medium containing nonradioactive and 14C-carbonyl-labeled chlorobromuron at a total concentration of 10 μg/ml, approximately 80% of the radioactivity initially present was still detected in the medium after 10 days of growth.

Ether extracts of the growth media were analyzed by TLC to determine the distribution of the remaining radioactivity. After 3 days of incubation, only 14% of the radioactivity initially present was found in the RF area of chlorobromuron. Concurrent with this disappearance was the appearance of new 14C-containing spots with RF values of 0.63 ("metabolite X") and 0.23 ("compound Y") (Fig. 1). In addition, small amounts of radioactivity were detected at the origin of the thin-layer plate and in the aqueous medium after ether extraction, but, due to the low levels of radioactivity, no attempt was made to characterize or identify these compounds.

![Fig. 1. Distribution of radioactivity after thin-layer chromatography during growth in culture medium of Rhizoctonia solani.](http://aem.asm.org/)

Downloaded from http://aem.asm.org/ on May 7, 2020 by guest
Metabolite X accumulated during the initial period of growth and 3 days after inoculation reached a maximum level, accounting for 67% of the radioactivity present in the growth medium; then it gradually decreased in concentration. This metabolite was isolated and purified by preparative TLC. Based on its chromatographic characteristics and breakdown pattern obtained by mass spectral analysis (Table 1), metabolite X was identified as 3-(3-chloro-4-bromophenyl)-1-methoxyurea, as the demethylated herbicide.

The spot with an $R_f$ value of 0.23 was designated compound Y, because it was determined that the production of this compound did not depend on the biochemical activity of the fungus, but was produced as a result of nonbiological phenomena acting on the demethylated herbicide. The chromatographic characteristics of this herbicide derivative did not coincide with any of the suspected and available metabolites of chlorbromuron. Equal amounts of compound Y were formed when the demethylated herbicide was incubated for 6 days at a concentration of 10 $\mu$g/ml in sterile media or growth media inoculated with R. solani. Mass spectral analysis indicated that compound Y had a molecular weight of 336 and possessed the characteristic breakdown pattern of a molecule containing one Cl and one Br atom (Table 1).

The ability of R. solani to transform the proposed metabolites of chlorbromuron was tested by growing the fungus in the presence of 10 $\mu$g of the following compounds per ml: 3-(3-chloro-4-bromophenyl)-1-methoxyurea (metabolite X); 3-(3-chloro-4-bromophenyl)-1-methylurea; 3-(3-chloro-4-bromophenyl) urea; and 3-chloro-4-bromoaniline. The pattern of fungal degradation of these compounds in comparison to sterile controls after TLC analysis is shown in Fig. 2. Chlorbromuron was completely transformed by the fungus to metabolite X and compound Y as well as a spot with an $R_f$ value of 0.29. This spot also appeared with the authentic demethylated herbicide after TLC and was therefore ignored. TLC analysis of the cultures containing the demethylated herbicide (metabolite X) revealed similar patterns of degradation in sterile and nonsterile samples; compound Y and an $R_f$ 0.29 spot appeared with equal intensity in both analyses. The demethoxylated herbicide remained intact during incubation in the sterile control, but was partially transformed to a compound with $R_f$ values equal to 3-(3-chloro-4-bromophenyl) urea. This urea derivative was partially degraded by R. solani, as indicated by reduction in size and intensity of the urea spot as compared with the sterile control.

3-Chloro-4-bromoaniline disappeared completely due to fungal activity. Since this compound appears only faintly under UV light, its transformation was followed by spraying with p-dimethylamino benzaldehyde as a chromogenic reagent. A weak UV-positive spot with

### Table 1. Chemical and physical characteristics of metabolite X, 3-(3-chloro-4-bromophenyl)-methoxyurea, and compound Y

| Substance | Thin-layer chromatography | Gas chromatography | Partial mass spectra bromine-chlorine clusters (p:p + 2:p + 4 + 100:130:032) |
|-----------|--------------------------|-------------------|---------------------------------------------------------------|
|           | $R_f$ value $^a$ | $R_f$ value $^a$ | Retention time | m/e | Relative intensities |
| Metabolite X | 0.42 | 0.63 | 2:57 | 278/280/282 | 0.3 |
| | 246/248/250 | 0.5 | 231/233/235 | 3.0 | |
| | 205/207/209 | 3.8 | 278/290/292 | 1.1 | |
| 3-(3-Chloro-4-bromophenyl)-methoxyurea | 0.42 | 0.63 | 2:57 | 246/248/250 | 0.4 |
| | 231/233/235 | 2.3 | 205/207/209 | 3.8 | |
| Compound Y | 0.28 | 0.23 | 2:02 | 336/338/340 | 0.5 |
| | 278/280/282 | 0.5 | 231/233/235 | 3.9 | |
| | 205/207/209 | 0.8 | |

$^a$ Ether-hexane (4:1, v/v).

$^b$ Methylene chloride-acetonitrile (4:1, v/v).
an \( R_f \) value of 0.78 appearing as a result of fungal growth does not react with the chromogenic reagent and was not investigated further.

Once it was established that \( R. \) solani is capable of degrading one of the phenylurea herbicides, its ability to transform related compounds was examined. The fungus was grown in the presence of 10 \( \mu \)g of the following compounds per ml: (i) chloroxuron; (ii) diuron; (iii) fenuron; (iv) fluometuron; (v) linuron; (vi) metobromuron; (vii) neburon; and (viii) siduron.

After growth of the fungus, the growth media were filtered, extracted, and examined by TLC analysis. The percentage of herbicide transformed was estimated by the size and intensity of the herbicide spot on the thin-layer plate relative to a sterile control (Table 2). In this preliminary study, no attempt was made to identify the resultant metabolites, but the use of \( p \)-dimethylamino benzaldehyde indicated that aniline intermediates did not accumulate.

**DISCUSSION**

Although the only clear evidence for the attack of chlorbromuron by \( R. \) solani is the isolation and identification of the demethylated herbicide, the data implicate the participation of the fungus in a more complete transformation of the herbicide as indicated by the loss of radioactivity from \( 1^C \)-carbonyl-labeled chlorbromuron. The ability of the fungus to transform the proposed metabolites of chlorbromuron also indicates a further transformation of the herbicide.

The demethylation of the demethoxylated herbicide, 3-(3-chloro-4-bromophenyl)-1-methylurea, to the corresponding urea can be expected, and there are also indications that the fungus converts the urea to the aniline. The complete and rapid disappearance of 3-chloro-4-bromoaniline provides an explanation as to why this probable metabolite was not detected in the culture media.

The appearance of compound Y has been shown to be independent of the biochemical activity of the fungus, but the production of compound Y from the demethylated herbicide may be of significance, if it results from the inherent instability of the metabolite.

The apparent ability of the fungus to attack a large number of phenylurea herbicides in spite of variations in ring structures, ring substituents, and alkyl moieties is notable. The fact that one organism is capable of attacking such a diverse group of substrates suggests that the fungus \( R. \) solani may possess a generalized ability to degrade this class of compounds. The data demonstrate that microbial degradation of chlorbromuron and a number of other phenylurea herbicides takes place and indicates the role of soil fungi in this process.

**LITERATURE CITED**

1. Alexander, M. 1961. Introduction to soil microbiology. John Wiley & Sons, Inc., New York.
2. Geissbühler, H., C. Haselbach, H. Aebi, and L. Ebner.
1963. The fate of N-(4-chlorophenoxy)-phenyl-N,N
dimethylurea (C-1983) in soils and plants. III. Break-
down in soils and plants. Weed Res. 3:277–297.
3 Hill, G. D., and J. W. McGahan. 1955. Further studies on
soil relationships of the substituted urea herbicides for
pre-emergence control. Proc. South. Weed Control
Conf. 8:284–293.
4 Hill, G. D., J. W. McGahan, H. Baker, D. W. Finnerty,
and C. W. Bingerman. 1955. The fate of substituted
urea herbicides in agricultural soils. Agron. J. 47:93–
104.
5 Katz, S. E., and R. F. Strusz. 1969. Gas chromatographic
separation of several urea herbicides and their metabo-
lites. J. Agr. Food Chem. 17:1409–1411.
6 Sheets, T. J. 1964. Review of disappearance of substi-
tuted urea herbicides from soil. J. Agr. Food Chem. 12:
30–33.
7 Tweedy, B. G., C. Loeppky, and J. A. Ross. 1970. Metabol-
olism of 3-(p-bromophenyl)-1-methoxy-1-methylurea
(metobromuron) by selected soil microorganisms. J.
Agr. Food Chem. 18:851–853.
8 Wallnöfer, P. 1969. The decomposition of urea herbicides
by Bacillus sphaericus, isolated from soil. Weed Res. 9:
333–339.