Metabolism of 3-(*p*-Bromophenyl)-1-methoxy-1-methylurea (Metobromuron) by Selected Soil Microorganisms

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A comparison was made of the metabolism of the herbicide, 3-(*p*-bromophenyl)-1-methoxy-1-methylurea (metobromuron) by four soil microorganisms, *Talaromyces wortmanii*, *Fusarium oxysporum*, *Chlorella vulgaris*, and a species of *Bacillus*. After 18 days incubation, the percent degradation by these microorganisms was 37, 11, 1, and 1, respectively. The metabolites produced by *T. wortmanii* were 1-(*p*-bromophenyl)-3-methoxy urea, 1-(*p*-bromophenyl)-3-methylurea, *p*-bromophenylurea, and *p*-bromoacetanilide. Identification was made by thin-layer chromatography and mass spectroscopy. It was established that *p*-bromoaniline was an intermediate in the degradation process and was rapidly and quantitatively converted to *p*-bromoacetanilide.

The increased addition of pesticides to soils has necessitated extensive investigations to determine the persistence of these compounds and their degradation products in the soil. Because of their diversity in phytotoxicity, the substituted urea herbicides are widely used, and numerous compounds belonging to this group have been developed for commercial use.

Microbial degradation of some substituted urea herbicides has been investigated. Hill *et al.* (1955) suggested that microbial degradation in the soil could potentially be a very important route for shortening the residual life of selected substituted urea herbicides, and they obtained evidence which suggested that the microbes could be utilizing monuron. The work of Sheets (1958) indicated that soil-borne microorganisms may play a very important role in residual phytotoxicity. He found that, of three substituted urea herbicides studied, all were more phytotoxic in autoclaved soils than in nonautoclaved soils. However, autoclaving alone changes many soil characteristics and it is possible that changes in soil characteristics could account for the increased toxicity.

Geissbuhler *et al.* (1963), on the basis of studies of mixed cultures of soil bacteria, have proposed a pathway for the degradation of chloroxuron. This pathway includes stepwise demethylation and deamination-decarboxylation.

Bozarth (1969) studied the degradation of ^{14}C-fluometuron in sandy loam soil. Fluometuron was degraded in nonautoclaved soil as indicated by the presence of ^{14}C-metabolites in the soil extracts and by the evolution of $^{14}CO_2$ from the treated soil. He suggested that the pathway of degradation involved a two-step demethylation followed by hydrolysis of the urea linkage to form the aniline derivative.

Geissbuhler (1969) showed that dealkylation and dealkoxylation of metobromuron occur in potatoes and corn seedlings. They identified 1-(*p*-bromophenyl)-3-methoxyurea, 1-(*p*-bromophenyl)-3-methylurea, and *p*-bromophenylurea as the major metabolites.

The formation of the aniline derivative followed by oxidation to form the azobenzene residues was first demonstrated by Bartha and Pramer (1967). In this initial study they found that 3′,4′-dichloropropionanilide is biodegraded in the soil to form aniline and azo derivatives. In a subsequent study, Bartha (1968) reported that the same compounds are formed from Dicryl and Kasil. In their earlier publication, they raised the question of the potential hazard which may exist in the formation of the azo compounds, since some are known to be carcinogenic. Kearney *et al.* (1969) recently reported that three different azo compounds were formed when 3,4-dichloroaniline and 3-chloroaniline were added to Nixon silt loam.

Kaufman and Miller (1969) reported that the aniline derivative is formed from several different substituted urea and carbamate herbicides. In some instances the corresponding azobenzene compounds were found, and in others the aniline was further degraded to carbon dioxide, water and the chloride ion. The proposed pathway for degradation is via demethylation, followed by subsequent hydrolysis of the urea. The desmethyl and aniline intermediates have been isolated, and the formation of carbon dioxide from the urea moiety has been demonstrated (Geissbuhler *et al.*, 1963). It was found that chloroxuron is degraded to the aniline compound via demethylation. Two additional compounds were not identified.

The objective of this investigation was to determine the metabolism of metobromuron by selected soil microbes, namely *Fusarium oxysporum* Schlect, *Talaromyces wortmanii*, *Chlorella vulgaris* Beijerinck, and *Bacillus sp.*

MATERIALS AND METHODS

The four soil microbes used in this study were *Fusarium oxysporum*, *Chlorella vulgaris*, a *Bacillus sp.*, and *Talaromyces wortmanii*. A liquid minimal synthetic medium (Gowans, 1960) was used for *C. vulgaris*, Burkholder's synthetic medium (1945) (pH 4.65) for the fungi, and a modification of Burkholder's (pH 6.8) for the bacterium. Metobromuron [3-(*p*-bromophenyl)-1-methoxy-1-methylurea] was ^{14}C ring-labeled, and was added aseptically to sterile media at a concentration of 10 μg/ml (0.1 μc/50 μg). Media were inoculated and incubated in a controlled environment chamber for 18 days at 24° C. Cultures were aerated by gyratory action at 200 rpm. Metabolites and parent compounds were extracted with chloroform. Percent recovery of total radioactivity added ranged from 92–98. The chloroform extract was added to a layered column of nonactivated florisil and charcoal. The metabolites and parent compounds were eluted with chloroform. Recovery from the column was nearly 100%. The eluants were concentrated and spotted on Brinkmann precoated glass tlc plates (silica gel type F-254) which had been developed with ethyl acetate before spotting. The solvent system used for separation of compounds was a 60 to 1 v/v mixture of chloroform and acetic acid. Autoradiograms were prepared by exposing the developed plates to x-ray film, and were used to locate the exact position of the radioactive metabolites on the thin-layer plates.

Identification of parent compounds and metabolites from *T.*

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Table I. Relative Quantities of Metobromuron and its Metabolites from *Talaromyces wortmanii*, as Extracted from Tlc Scrapings

Spot	R_f Value	Total radioactivity[a] isolated (cpm × 10^{-3})	% of Total	Total μmoles	Total μg
1	0.09	166	7.8	0.326	70
2	0.18	114	5.3	0.273	51
3	0.28	167	8.0	0.329	70.3
4	0.41	334	15.8	0.657	161.0
5	0.54	1336	63.1	2.626	680.4

[a] Sp. Act. of metobromuron: 1 μc/mg; 1 mmole = 259 μcuries.

Table II. Partial Mass Spectra of Metobromuron Metabolites from *Talaromyces wortmanii*, Isolated by Tlc

m/e	Relative Intensities[a] Spot 1	Spot 2	Spot 3	Spot 4[b]
171	100	100	100	46
173	97	96	97	40
197	11	8	...	98
199	11	7	...	100
213			21	
215			21	
214	14			
216	14			
228		13		
230		13		
244				33
246				33

[a] Relative intensities are normalized relative to the highest peak in the high mass region of the spectra. [b] Peak at *m/e* 47 has intensity of 600 relative to the *m/e* 199 peak.

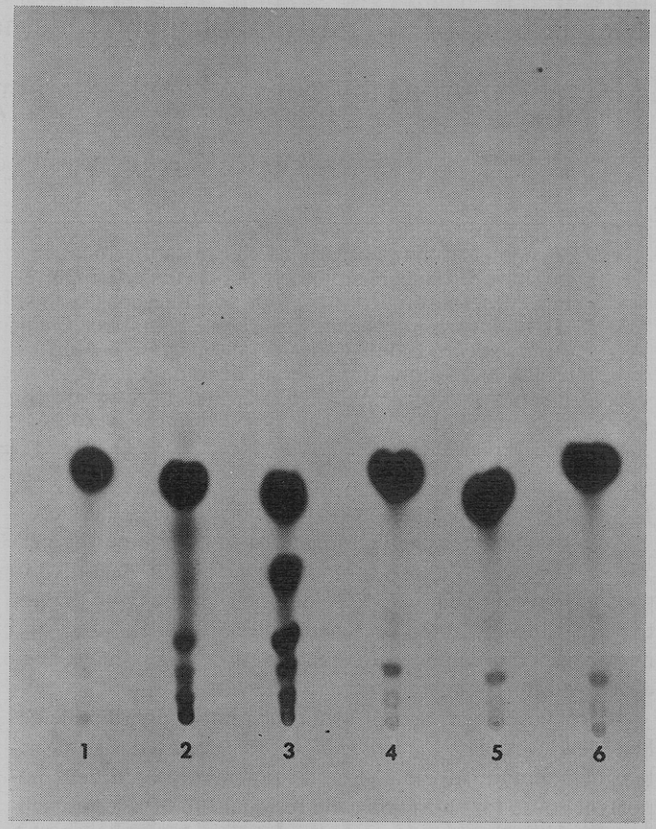


Figure 1. Degradation of metobromuron by selected soil-borne microorganisms. (1) *standard compound* (2) *Fusarium oxysporum* (3) *Talaromyces wortmanii* (4) *Bacillus sp.* (5) *Chlorella vulgaris* (6) *uninoculated control*

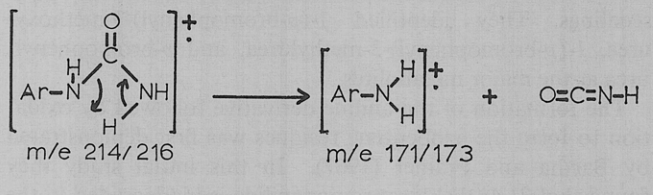
wortmanii was accomplished by mass spectrometry and thin-layer chromatography. Metabolites were isolated for mass spectrometric analysis by extracting individual tlc spots, showing the major amounts of radioactivity with 10–22 ml of ethyl acetate. An aliquot of each extract was counted in a liquid scintillation counter, and the total radioactivity of each spot was calculated (Table I). The remaining ethyl acetate solutions were concentrated under reduced pressure and chromatographed on Sephadex LH-20 using ethyl acetate as the eluent. The fractions showing radioactivity were concentrated and then dried in quartz capillaries designed to accommodate the direct probe of a Perkin-Elmer Model 270 GC-DF mass spectrometer. The mass spectra of the samples were obtained at 2000 v accelerating voltage and 70 eV ionizing voltage. The samples in the quartz capillaries were introduced by means of the solid inlet probe operated at *ca.* 75° C with a housing temperature of 150° C.

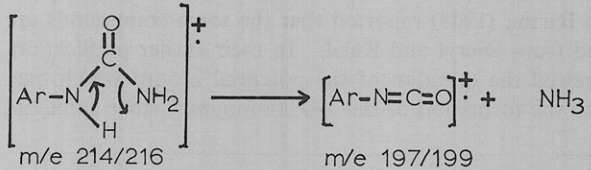
RESULTS

Degradation by Microorganisms. A comparison of degradation of metobromuron by the four microorganisms after 18 days incubation can be made by observing the autoradiogram shown in Figure 1. *T. wortmanii* degraded 37% of the total metobromuron added, *F. oxysporum* 11%, and both *C. vulgaris* and an unidentified bacillus (*B. sp.*) less than 1%. Because it produced the greatest quantities, *T. wortmanii* was chosen for use in identification of metabolites of metobromuron.

Identification of Metabolites. An autoradiogram of the radioactive metabolites of metobromuron produced by *T. wortmanii* and the calculated R_f values are shown in Table I. The relative quantities of each metabolite, as determined from tlc scrapings, are recorded in Table I. Purification of tlc scrapings for mass spectral analysis involved ethyl acetate extraction followed by Sephadex chromatography to remove remaining yellow impurities. The mass spectra still showed significant residual impurities which contributed to high background peaks in the spectra. Bromine isotope clusters, however, were readily distinguished in the mass regions above *m/e* 170, and no difficulty was encountered in detecting the molecular ions and important fragmentation ions at *m/e* 171/173 and 197/199. The partial mass spectra of the extracted tlc spots of metobromuron metabolites are given in Table II.

Spot 1. This metabolite has the same R_f value as *p*-bromophenylurea, and the molecular ion at *m/e* 214/216 confirms this assignment. The peaks at *m/e* 171/173 and 197/199 are the results of the following fragmentations.



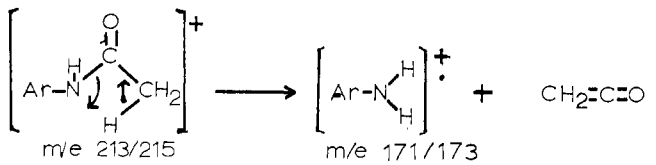


Undoubtedly a high proportion of these fragmentations is thermal and not due to electron impact, since the relative

intensities vary with the source temperature and the fragment ions are apparent long after the parent compound has been baked out of the spectrometer.

Spot 2. This material is identified as 1-(*p*-bromophenyl)-3-methylurea by its molecular ion at *m/e* 228/230. The fragmentation to the *m/e* 171/173 and 197/199 ions is completely analogous to *p*-bromophenylurea.

Spot 3. This compound is found to be *p*-bromoacetanilide by its molecular ion at *m/e* 213/215 and its chromatographic mobility, which is identical to that of an authentic sample. In this case the *m/e* 197/199 peaks are missing since the compound cannot undergo the fragmentation and rearrangement to *p*-bromophenylisocyanate. Loss of ketene by the route shown below provides the observed high abundance of *p*-bromoaniline ions.



Spot 4. This metabolite has an R_f value identical to that of authentic 1-(*p*-bromophenyl)-3-methoxyurea. This assignment is confirmed by its mass spectrum which exhibits molecular ions at *m/e* 244/246.

Of the four microorganisms, two (*T. wortmanii* and *F. oxysporum*) produced the acetanilide from metobromuron and two (*C. vulgaris* and bacillus) did not. All of the organisms, however, rapidly converted *p*-bromoaniline at concentrations of 1, 10, and 20 μg per ml to the acetanilide (100% in 3 to 10 days, depending on the organism). The detection of *p*-bromoaniline in these and other experiments using the pure compound with the chloroform-acetic acid tlc solvent system discounts the possibility that acetylation occurs during the chromatography. In no case could the conversion of the aniline to the acetanilide be detected under the tlc conditions.

DISCUSSION

The metabolic scheme outlined in Figure 2 provides a rationale linking the various isolated metabolites of metobromuron. The demethylation steps A and A′ are analogous to demethylations described for several other substituted ureas and probably involve initial oxidations of the methyl groups to C-hydroxy derivatives (not isolated) which are hydrolyzed to the observed products. Processes B and B′ are reductive cleavages of the N—O bond (hydrolytic cleavage would give the *N*-hydroxy compounds).

The absence of the aniline derivative in the ambient solution suggests that either *p*-bromoaniline is not an intermediate or it is rapidly converted to the acetanilide, allowing insufficient quantities for detection. To determine which of these suppositions was most likely occurring, *p*-bromoaniline at concentrations of 1, 10, and 20 μg/ml was incubated with each of the above microorganisms. All four quantitatively converted the *p*-bromoaniline to the acetanilide after an incubation period of 7 days. These results suggest that acetylation of the aniline derivative could be quite prevalent among the soil microorganisms. These results further indicate that the bacterium and algae species lack the enzyme system necessary to convert metobromuron to *p*-bromoaniline.

Although the metabolism of metabromuron is shown as a stepwise demethylation and demethoxylation to the *p*-bromophenylurea and hence to the aniline, it is conceivable that *p*-bromoaniline can be formed by direct hydrolysis of metobromuron or any of the intermediate products. The relative rates of such reactions are now being studied to determine the precise pathway of metabolism. The aniline derivative is then acetylated to provide *p*-bromoacetanilide by a fast process which does not allow the accumulation of the free aniline in the system. This supposition is confirmed by the rapid conversion of *p*-bromoaniline to the acetanilide.

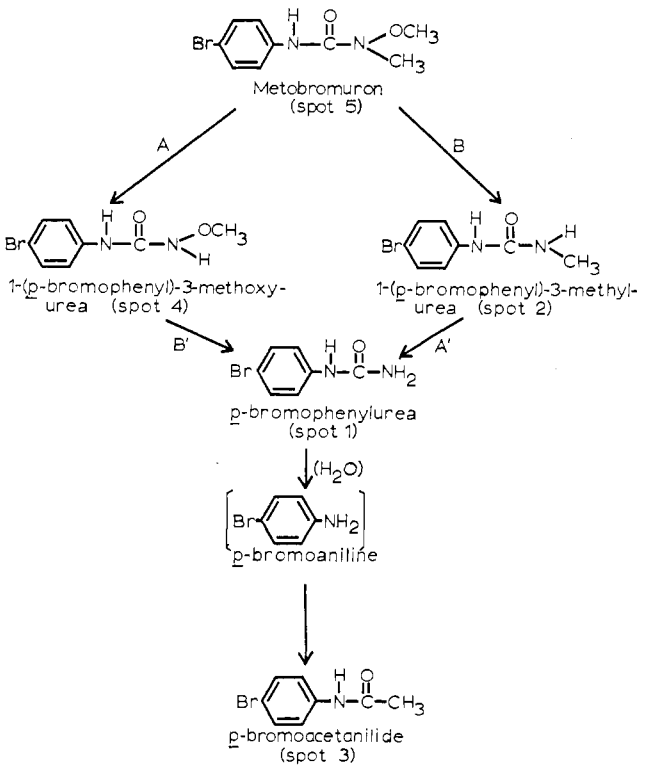


Figure 2. Proposed scheme for the metabolism of metobromuron by *Talaromyces wortmanii*

In view of the reports of Bartha and Pramer (1967), we were especially interested in determining whether or not any of the aniline derivative was oxidized to form 3,3′-dibromoazobenzene. In no case was there any evidence that this compound was formed. The data presented in this paper suggest that acetylation of substituted anilines in soils could be a major factor in preventing accumulation of the aniline derivatives and also could be competitive with the oxidative coupling to the azobenzenes, at least in the case of the bromine-containing aniline from the phenylureas.

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