METABOLISM OF FLUORODIFEN BY SOIL MICROORGANISMS

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Summary—The metabolism of fluorodifen (p-nitrophenyl 2,2,2-trifluoro-2-nitro-p-tolyl ether) by soil microorganisms in the presence or absence of other carbon and nitrogen sources was studied. The degradation of this herbicide continued for 5 days, when benzoate or acetate and ammonium sulphate were included in the cultures, and for more than 5 weeks when fluorodifen was used as a sole source of carbon and nitrogen.

Under all conditions nitrite ions were produced at concentrations ranging between 5 and 80 per cent of the nitro-nitrogen of the fluorodifen present. The highest concentration of nitrite was obtained when added carbon sources were used with fluorodifen. The lowest nitrite concentration accumulated when the fluorodifen was used as sole source of carbon and nitrogen. The nitrite reached a maximum value after a few days of incubation, followed by rapid disappearance.

p-Nitrophenol and quinol were identified in the acid-ether extract of cultures. It is suggested that the first step in the degradation of fluorodifen is the hydrolysis of the ether linkage followed by the direct elimination of the nitro-groups as nitrite ions.

INTRODUCTION

Many nitro-compounds are used in agriculture as fungicides, insecticides and herbicides. Their degradation in soil is of great importance to ensure the safety of subsequent crops, and to maintain the activity of soil organisms. Adsorption, leaching, chemical decomposition and photochemical decomposition may play only a small part in the detoxication of many of these agrochemicals. Microbial degradation is a major factor in the disappearance and transformation of these compounds. The metabolism of fluorodifen (p-nitrophenyl 2,2,2-trifluoro-2-nitro-p-tolyl ether) in higher plants has been reported earlier (Eastin, 1969, 1971). The fate of this herbicide in soil, however, has not been fully investigated. The aim of the present communication is to study the metabolism of fluorodifen by soil microorganisms.

MATERIALS AND METHODS

Media

The following mineral salts medium containing K$_2$HPO$_4$, 0.5; MgSO$_4$.7H$_2$O, 0.5; NaCl, 0.1; CaCl$_2$.5H$_2$O, 0.1; and Fe$_2$SO$_4$.7H$_2$O, 0.05 g/l, was used as a basal medium. To this medium different compounds were added as carbon and nitrogen sources as presented in Table 1.

Pure crystalline fluorodifen (prepared from the commercial herbicide known as “Preforan”, 35% active ingredient) was used in the cultures at the rate of 50 µmoles/100 ml of media dissolved in 0.1 ml of ethanol (95%).

Cultural conditions

Silt loam soil leachate (100 g in 900 ml of basal medium) was added to each of 100 ml of media at the rate of 20 g before the addition of the herbicide and the other carbon and nitrogen sources (Table 1). The media were dispensed in 250 ml flasks to a depth of 2.5 cm. Cultures were then incubated at 25°C.

Estimation of fluorodifen

Samples of 10 ml of the incubated cultures were acidified to pH 3 with H$_2$SO$_4$ (0.1 n) then extracted three times with ether and the combined ether extract was dried over anhydrous Na$_2$SO$_4$. The solvent was then evaporated under reduced pressure and the residues were dissolved in 10 ml ethanol (95%, v/v). The fluorodifen concentration was estimated by the absorbance at 290 nm.

Table 1. Composition of different media used in the metabolism of fluorodifen

<table>
<thead>
<tr>
<th>Code number of medium</th>
<th>Carbon source (3.0 g/l)</th>
<th>Nitrogen source (1.0 g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na acetate</td>
<td>(NH$_4$)$_2$SO$_4$</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>(NH$_4$)$_2$SO$_4$</td>
</tr>
<tr>
<td>3</td>
<td>Na benzoate</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Na acetate</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Na acetate</td>
<td>(NH$_4$)$_2$SO$_4$</td>
</tr>
<tr>
<td>6</td>
<td>Na benzoate</td>
<td>(NH$_4$)$_2$SO$_4$</td>
</tr>
</tbody>
</table>
Estimation of nitrite ions

Samples of 1 ml of the cultures were used to estimate nitrite employing Griess reagent and the absorbance at 525 nm was measured on a Unicam Spectrophotometer.

Determination of phenols

The total phenols produced in the cultures were estimated with Folin and Ciocalteu reagent (Smith, 1969).

Chromatography

Paper chromatography was used for the separation of fluorodifen intermediates on Whatman No. 1 papers. Authentic samples of the expected intermediates were run simultaneously with the culture extracts. The following solvents were used: (i) sodium formate (5%, w/v solution in water):formic acid (200:1); (ii) isopropanol:ammonia:water (100:5:10).

The following reagents were used for the detection of phenolic intermediates: (i) Folin–Ciocalteu reagent (BDH Ltd., Poole, England) used undiluted, followed by 10%, Na₂CO₃ or diluted 1:3 and developed with NH₃ vapour; (ii) diazotized p-nitroaniline; and (iii) diazotized sulphuric acid (Smith, 1969). For the detection of dihydroxy compounds, ammonical silver nitrate reagent (Smith, 1969) was used.

RESULTS

The degradation of fluorodifen varied greatly according to the medium used. In the presence of fluorodifen as a carbon and nitrogen source, the degradation of 50 μmoles (0.5 μmole/ml of medium) of the herbicide took 5 weeks (Fig. 1a). In the presence of acetate or benzoate and ammonium sulphate, it was complete in 5 days (Fig. 1e and f). When the fluorodifen was used as a sole source of added carbon or nitrogen, it persisted for 3 weeks (Fig. 1b) or approximately 1 week (Fig. 1c and d), respectively. In all treatments, only a very short lag or none was noticed.

The production of nitrite ions was observed in all cultures, however, the concentration varied according to the different media. Maximum nitrite production occurred within the first few days followed by rapid disappearance. This is probably due to the induction of nitrite reductase in the organisms present. Thus in cultures (Fig. 1a and b) the nitrite concentration reached only 5–10 per cent of the theoretical maximum. In cultures where the degradation of fluorodifen was very rapid, however, the nitrite concentration reached 75–80 per cent of the theoretical nitro-nitrogen before its disappearance (Fig. 1e and f).

In the presence of acetate and benzoate as an additional carbon source with fluorodifen as a nitrogen source (Fig. 1c and d), intermediate levels of nitrite of 25–35 per cent were produced.

The phenolic intermediates in cultures were estimated as quinol. As illustrated in Fig. 1, the accumulation of phenolic compounds started after the initiation of fluorodifen degradation. In most cases, it reached its maximum concentration after the nitrite ions had reached their maximum value.

In some instances, especially in the presence of other sources of carbon and nitrogen, the phenolic intermediates reached a value between 1.5 and 1.65 mole/mole of the original substrate.

The fluorodifen intermediates are presented in Table 2. Compounds (d) and (f) were identical with p-nitrophenol and quinol, respectively. In each case, both the culture and the authentic compound had the same Rf values in different solvents and developed the same colour with the different spray reagents. No p-amino phenol was detected in any of the extracts, indicating no reduction of the nitro-group.

DISCUSSION

The microbial degradation of nitro-compounds is known to proceed through two different pathways.
One mechanism is to eliminate the nitro-group as nitrite ions which accumulate in the culture medium or are rapidly reduced to ammonia. The alternative is to reduce the nitro-group to an amino-group before its elimination (Tewfik and Evans, 1966). Organisms seem to possess a single mechanism to remove the nitro-group, regardless of the medium used (Tewfik, 1966). A few organisms, however, possess the ability to use the two mechanisms. Thus, Nocardi a sp. strain M eliminated the nitro-group directly from m-nitrobenzoate to produce m-hydrobenzoic acid (Cartwright and Cain, 1959a), but in a very rich medium it was able to produce m-aminobenzoate (Cartwright and Cain, 1959b). The latter intermediate, however, was not on the direct metabolic pathway. Similarly, a pseudomonad strain capable of degrading trifluralin (2,6 dinitro-N,N-dipropyl-4-trifluromethylaniline) (Hamdi and Tewfik, 1969) eliminated the nitro-group as nitrite ions when trifluralin was used as a sole source of carbon, and used the reductive pathway when other organic substrates such as glutamate or acetate were used in the cultures (Hamdi et al., 1969).

In the present study, nitrite was produced in all cultures even in the highly enriched media, indicating that the presence of acetate or benzoate had no influence on changing the mechanism by which the nitro-group is degraded. That the nitrite level reached as high as 1.6 mole/mole of herbicide estimated as quinol (i.e. the production of 2 moles of dihydroxy phenolic compounds/mole substrate) indicates the hydrolysis of the ether linkage of fluorodifen (I). That hydrolysis occurs before elimination of nitrite, is supported by the identification of p-nitrophenol (II) in the culture extract. It is clear from Fig. 1, however, that hydrolysis occurs before elimination of nitrite, is supported by the identification of p-nitrophenol (II) in the culture extract. It is clear from Fig. 1, however.

![Scheme 1. Suggested pathway of fluorodifen degradation.](image-url)

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### Table 2. R<sub>f</sub>-Values and reaction with different reagents of fluorodifen intermediates and authentic compounds

<table>
<thead>
<tr>
<th>Intermediates</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;-values</th>
<th>Folin-Ciocalteu</th>
<th>dpna*</th>
<th>dsa†</th>
<th>AgNO&lt;sub&gt;3&lt;/sub&gt; (ammon)</th>
<th>NH₃ vapour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent (i)</td>
<td>Solvent (ii)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>0</td>
<td>0.22</td>
<td>+</td>
<td>faint brown</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>0</td>
<td>0.48</td>
<td>+</td>
<td>violet</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>0</td>
<td>0.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(d)</td>
<td>0.48</td>
<td>0.65</td>
<td>-</td>
<td>yellow</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(e)</td>
<td>0.70</td>
<td>0.91</td>
<td>++++</td>
<td>faint brown</td>
<td>+</td>
<td>brown</td>
</tr>
<tr>
<td>(f)</td>
<td>0.78</td>
<td>0.35</td>
<td>++++</td>
<td>faint brown</td>
<td>+</td>
<td>brown</td>
</tr>
<tr>
<td>Authentic compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorodifen</td>
<td>0</td>
<td>0.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>p-Nitrophenol</td>
<td>0.43</td>
<td>0.65</td>
<td>-</td>
<td>yellow</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Quinol</td>
<td>0.78</td>
<td>0.35</td>
<td>++++</td>
<td>faint brown</td>
<td>+</td>
<td>brown</td>
</tr>
</tbody>
</table>

* dpna = Diazotised p-nitroaniline.
† dsa = Diazotised sulphanilic acid.

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**Metabolism of fluorodifen**

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that the nitrite ions reach their maximum concentration before the phenolic intermediate. This may appear contradictory to the above, but can be explained by the fact that hydrolysis of fluorodifen will give rise to 2 moles of nitrophenol derivatives (II and III) which do not react with Folin Ciocalteu reagent until after the elimination of the nitro-group. Quinol (IV) is probably further oxidized by ring fission in a similar way to that described by Larway and Evans (1965).

The fate of the trifluoromethyl group of 4-trifluoromethyl catechol (V), however, is debatable. One possibility is that the ring is opened while it is still bearing the unchanged trifluoromethyl group. The other alternative is a reductive or oxidative dehalogenation of fluorine to give 4-methyl catechol or protocatechuic acid respectively before ring cleavage. Further investigation is necessary to clarify this point.

The present work indicates that fluorodifen in soil suspensions has a moderate persistence. In soil rich in organic matter and nitrogen content, it might last only for a very short time.

REFERENCES