Degradation of the Herbicide Molinate in Soils

Yasufumi IMAI* and Shozo KUWATSUKA

Laboratory of Soil Science, Faculty of Agriculture, Nagoya University,
Furo-cho, Chikusa-ku, Nagoya 464, Japan

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Laboratory experiments were conducted to study the degradation of molinate (S-ethyl hexahydro-1H azepine-1-carbothioate) in three soils of different types under upland and flooded conditions using two kinds of 14C-molinate (labelled at S-ethyl-l-C and azepine ring-2-C, respectively) and non-labelled molinate. The degradation rates of molinate were more rapid and the 14CO2 evolution was greater under upland conditions than under flooded conditions. The rates of degradation varied largely among the soils. In sterile soils, disappearance of molinate was very slow compared to the unsterile soils. Several degradation products of molinate were identified by tic, GC and GC-MS. These were molinate-sulfoxide, azepine ring-4-hydroxy- and 4-oxo-derivatives, S-carboxyl methyl derivative and hexamethyleneimine which have been already reported, and azepine ring-2-oxo-derivative, S-2-hydroxyethyl derivative and ethane sulfonate which were newly recognized. The degradation pathways which contained oxidation of sulfur atom, azepine ring and S-ethyl moiety, and hydrolysis to yield hexamethylene imine and/or ethane sulfonate are proposed. Main degradation pathways were ring oxidation under upland conditions, and oxidation of S-ethyl moiety under flooded conditions. The relationship between molinate degradation and soil properties was also discussed.

INTRODUCTION

The herbicide molinate (S-ethyl hexahydro-1H-azepine-1-carbothioate) is widely used for the control of barnyard grass in paddy fields. A few studies have been done to elucidate the behavior of this herbicide in the environment. Studies on the microbial degradation of molinate in pure cultures,1,2) its photodegradation and volatilization,3) and its persistence in paddy fields4,5) and river water6) have already been reported. Thomas et al.7) also recently reported the molinate degradation in a soil under flooded and nonflooded conditions.

In this study, the degradation rates and products in three soils were compared under upland and flooded conditions. The relationship between the degradation rates and soil conditions coincided with the results of the former studies.7) However, some of the degradation products reported by Thomas et al.7) were not detected, and several other intermediates were identified.

MATERIALS AND METHODS

1. Radioactive Molinate

Ring-14C-molinate (S-ethyl-2-14C-hexahydro-1H-azepine-1-carbothioate) and ethyl-14C-molinate (S-1-14C-ethyl hexahydro-1H-azepine-1-carbothioate) which were synthesized in the Radiochemical Centre, Amersham, United Kingdom, were gifted from Asahi Chemical Industry Co., Ltd. Specific radioactivities were 12.7 and 2.9 mCi/mmol respectively, and radiochemical purities were 98 and 99% respectively.
2. Chemicals
Molinate was synthesized by reacting hexahydro-1H-azepine-1-carbothioate hexamethylene imine salt (HMI-thiocarbamate—HMI salt) with monochloroethane in benzene and redistilled twice under reduced pressure. Its purity was 99% based on the area ratio of a TCD gas chromatogram.

Molinate sulfoxide, 1-[(ethylsulfinyl)carbonyl]hexahydro 1H-azepine, mp. 34.5–35.5°C; molinate sulfone, 1-[(ethylsulfonyl)carbonyl]hexahydro-1H-azepine-1-carbothioate, viscous oil, MS: m/z 201 (M⁺), 172 (C₆H₁₀N₀₂S), 140 (base, C₆H₁₀N₀₂O) were synthesized according to Soderquist et al. HMI-thiocarbamate—HMI salt, mp. 114.5–115.5°C was synthesized from 1 mol of hexamethylene imine (HMI) in 300 ml benzene into which 60 g of COS gas was blown for 2 hr at 5–10°C, and recrystallized from n-hexane-acetone. Carboxyl derivative of molinate (molinate-acid), S-carboxyl methyl hexahydro-1H-azepine-1-carbothioate mp. 83.9–84.1°C, was synthesized by reacting HMI-thiocarbamate—HMI salt with monochloroacetate in benzene and recrystallized from benzene. S-2-Hydroxy-ethyl derivative of molinate (molinate-alcohol), S-2-hydroxy-ethyl hexahydro-1H-azepine-1-carbothioate, viscous oil, MS: m/z 203 (M⁺), 185 (C₆H₁₂NOCOSCH₂CH), 184 (C₆H₁₂NOCOSCH₂C), 172 (C₆H₁₂NOCOSCH₂), 158 (base, C₆H₁₂NOCO) was synthesized by reacting HMI-thiocarbamate—HMI salt with 2-bromoethanol in dimethylacetamide and refined in a silica gel column. 4-Hydroxy hexamethylene imine (4-OH-HMI), viscous oil, MS: m/z 115 (M⁺), 98 (C₆H₁₀NH), 97 (C₆H₁₀NH), was synthesized according to Yokoo et al. and Morosawa. 4-Hydroxy molinate (4-OH-molinate), S-ethyl hexahydro-4-hydroxy-1H-azepine-1-carbothioate, viscous oil, MS: m/z 203 (M⁺), 174 (C₆H₁₂ONCOS), 142 (base, C₆H₁₂ONCO), was synthesized from 4-OH-HMI and ethylchlorothioformate in benzene and refined in a silica gel column. Methyl ester of molinate acid, viscous oil, MS: m/z 231 (M⁺), 200 (C₆H₁₂NOCOSCH₂CO), 158 (C₆H₁₂NCO), 126 (base, C₆H₁₂NCO), was synthesized from HMI-thiocarbamate—HMI salt and methyl-monobromoacetate in dimethylacetamide and refined in a silica gel column.

Molinate, HMI-thiocarbamate—HMI salt, molinate acid, molinate alcohol, 4-OH-HMI, and methyl ester of molinate acid were provided by Asahi Chemical Industry Co., Ltd.

3. Soil Sample
Three arable soils, Anjo, Nagano and Tochigi, were used. They were selected as a diluvial soil of kaolinite clay mineral (Anjo), an aluvial soil of montmorillonite clay mineral (Nagano), and a humic volcanic ash soil (Tochigi), respectively. These soils were taken from the plough layers of the paddy fields in winter and the undried samples were crushed to pass through a 2 mm sieve.

The characteristics of these soils were as follows: Anjo soil: mineral soil with kaolinite clay, SCL, clay content 23.1%, pH(H₂O) 5.4, total carbon (T-C) 1.96%, total nitrogen (T-N) 0.142%, pH(H₂O) 5.4, total carbon (T-C) 1.96%, total nitrogen (T-N) 0.142%, C.E.C. 10.0 mEq/100 g, maximum water holding capacity (MWHC) 56.3%; Nagano soil: mineral soil with montmorillonite clay, CL, clay content 20.5%, pH 5.9, T-C 1.52%, C.E.C. 25.7 mEq/100 g, MWHC 63.0%; Tochigi soil: humic volcanic ash soil with allophane clay, SiL, clay content 5.4%, pH 4.9, T-C 10.3%, C.E.C. 46.7 mEq/100 g, MWHC 95.7%.

4. Soil Conditioning
Upland conditions: Anjo soil (50 g dry weight), Nagano soil (50 g dry weight), or Tochigi soil (25 g dry weight) was put into 200-ml Erlenmeyer flasks and water was added to the flasks to 60% of maximum water holding capacity. The flasks were capped with aluminum foil and preincubated at 30°C for 2 weeks in the dark. Water lost was made up when necessary.

Flooded conditions: The same amount of soils as above was put into 300-ml Erlenmeyer flasks and water was added to the flasks to 60% of maximum water holding capacity. The flasks were capped with aluminum foil and preincubated at 30°C for 2 weeks in the dark. Water lost was made up when necessary.

5. Application and Incubation of ¹⁴C-molinate
The soils were added with 0.5 ml of ring-¹⁴C-molinate acetone solution (10 ppm molinate
in dry soil basis, $1.25 \times 10^4$ dpm) and mixed well with a glass rod. A small vessel containing 3 ml of 5 N NaOH was placed in each flask to trap CO$_2$ evolved. The flask was incubated under the same conditions as described for preincubation.

Anjo soil was also added with 0.5 ml of ethyl-$^{14}$C-molinate acetone solution (10 ppm molinate in dry soil basis, $1.66 \times 10^6$ dpm) and incubated as above to compare the transformations of 2 different positional carbons in molinate.

6. Determination of $^{14}$CO$_2$

$^{14}$CO$_2$ evolution from the soil was determined periodically during the incubation. The trap solution in the small vessel in the flask was renewed twice a week. The small aliquot of this solution was radioassayed using an alkali scintillator$^{10}$ and the radioactivity was measured by an Aloka 671 liquid scintillation spectrometer. The details were described previously.$^{10}$

7. Extraction and Fractionation of Radioactive Compounds

Fifty milliliters of water plus 1 ml of 10 N H$_2$SO$_4$ (upland conditions), or 1 ml of 10 N H$_2$SO$_4$ alone (flooded conditions) was added to the flask to transfer CO$_2$ in the soil water into the trap solution in the small vessel, and then the contents were transferred to a 300 ml-centrifuge tube. To the contents in the tube 150 ml of methanol was added, and the tube was capped tightly, shaken at 400 rpm for 30 min, and centrifuged at 2,000 g x 15 min. The supernatant was transferred into a flask and the sedimented soil was extracted once with 200 ml of 75% methanol and then twice with 200 ml of 1 N NaOH·MeOH (1 : 3).

The supernatant solutions were combined, adjusted to pH 3 with conc. HCl and extracted three times with $n$-hexane (hexane fraction). The residual solution was concentrated under reduced pressure to about 500 ml, adjusted to pH 10 with 10 N NaOH, and extracted with ether three times (basic ether fraction). The residual solution was then adjusted to pH 1 with conc. HCl and extracted with ether three times (acidic ether fraction). Small aliquots of each fraction were radioassayed using a dioxane scintillator.$^{11}$ The residual soil after methanol extraction was radioassayed by the wet combustion method.$^{13,14}$

8. Thin Layer Chromatography (tlc)

The hexane and ether extracts were dried with anhydrous Na$_2$SO$_4$ and concentrated. An aliquot of each concentrate was subjected to two dimensional tlc using silica gel plates, Merck 60 F$_{254}$, precoated, 0.25 mm thick, 20 x 20 cm. Authentic compounds (Table 1) were used for identification of radioactive spots.

For the hexane and basic ether fractions, solvent A was used for the first development and solvent B for the second, while for the acidic ether fraction, solvent C was used for the first and solvent B for the second. Spots of the authentic compounds on the plate were detected by irradiation of UV light (254 nm) or exposure in I$_2$ vapor. The radioactive spots were detected by radioautograms on X-ray films (Medical X-ray film Kx, Fuji Film).

The radioactive spots on the tlc plate were scraped and extracted with methanol. Each aliquot of the extract solutions was radioassayed in a toluene scintillator (PPO 4 g, POPOP 0.1 g in 1 l toluene). If necessary, these extracts were again subjected to cochromatography with authentic compounds using solvents D, E or F.

9. Confirmation of the Structures of Degradation Products using GC and GC-MS

In order to identify the degradation products which did not correspond with any authentic compounds by tlc and also to confirm the results of tlc analysis, degradation products from unlabelled molinate were analyzed by GC and/or GC-MS. Non-labelled molinate was mixed into 200 g of Anjo soil at 25 ppm and incubated for 10 days under upland conditions or 40 days under flooded conditions. The degradation products were extracted and fractionated according to the method described earlier. Each of the hexane or ether extracts was spotted on the tlc plate (0.5 mm thick) and the corresponding extract from the soil mixed with $^{14}$C-molinate and incubated was spotted beside these spots. The plate was developed with the solvent A
(hexane or basic ether fraction) or solvent C (acidic ether fraction). The radioactive zones and the corresponding zones of unlabelled products were scraped and extracted with methanol. Each of these extracts was again subjected to the same TLC process using solvent B. Thus the unlabelled degradation products corresponding to the radioactive spots on the two dimensional TLC plates were obtained. These products were analyzed by GC with reference compounds using a Hewlett Packard model 5715, NP-FID detector, 2mm i.d. x 100 cm glass column packed with OV-17 1.5%/chromosorb W (AW-DMCS) 60/80 mesh, Wako Pure Chemical Co., Ltd., at 150-200°C of column temperature; or packed with PEG 6000-KOH 10%-10%/chromosorb W 60/80 mesh, Nihon Chromato Works Ltd., at 70-150°C of column temperature. Some of the products were analyzed directly by GC-MS using a JEOL-D 100 mass spectrometer attached directly to a JEOL-20 K gas chromatograph (carrier: He, detector: total ion monitor, chamber temperature 290°C, ionizing current 300 µA, ionizing energy 30 eV, column condition: the same as GC), and other products were analyzed after methylation with diazomethane or reduction with NaBH₄.

10. Determination of ¹⁴C-ethane Sulfonate

¹⁴C-ethane sulfonate produced from ethyl-¹⁴C-molinate in the soils was determined. One hundred milliliters of the residual aqueous solution which remained after extraction with acidic ether was passed through anion exchange resin (50 ml of Dowex WGR, OH form) in a column (φ=1.5 cm). The column was washed with 500 ml of water, and eluted with 200 ml of 15 N NH₄OH-MeOH (3 : 2). The eluate was concentrated to dryness and dissolved in 50 ml of water. This solution was passed through a column packed with 50 ml of Dowex HCR (H⁺ form) and then 200 ml of water was passed through the column. By this process, the solute was changed from the ammonium salt form to free acid form. This eluate was concentrated to dryness, dissolved in a small amount of methanol, spotted on a
cellulose thin layer plate (Merck, precoated, cellulose F, 0.1 mm thick), and developed with solvent G together with standard ethane sulfonate. Ethane sulfonate was detected as a white spot on a brownish background by $I_2$ vapor. The radioactive spots were detected by radioautogram, scraped and the radioactivity was determined in the mixture of 1 ml of methanol and 10 ml of dioxane scintillator. The recovery of ethane sulfonate in this process was 60–70% when the authentic chemical on the developed tlc plates was extracted with methanol, methylated by diazomethane, and then analyzed by GC (the same model as described before, FID detector, 2 mm i.d. × 100 cm glass column packed with OV-17 1.5%, column temperature 75°C).

11. Degradation in the Sterile Soils

The three soils were preincubated as described before, and autoclaved three times intermittently at 120°C for 30 min. Unlabeled molinate was added to each soil at 10 ppm (dry soil basis). After incubation for designated periods, the soils were extracted by the same procedure as described before and molinate in the hexane fraction and the basic ether fraction was determined by GC (NP-FID) as described before.

RESULTS

1. Degradation of Molinate in the Three Soils under Upland and Flooded Conditions

As shown in Fig. 1, most of the radioactivity in the methanol extract was attributed to $^{14}$C-molinate except in Nagano soil under flooded conditions. $^{14}$C-molinate was degraded more rapidly under upland conditions than flooded conditions in all soils, although the rates of degradation varied among these three soils. The half-lives of molinate were 8–25 days under upland conditions and about 40–160 days under flooded conditions.

The radioactivity in the alkaline trap solution was attributed only to $^{14}$CO$_2$, because this radioactivity disappeared by acidifying this solution with HCl and no radioactivity was detected in the supernatant after adding 1 M BaCl$_2$ to this solution. When ethyl-$^{14}$C molinate was added to the soil, all the radio-

![Fig. 1 Changes in radioactivity of fractions from different soils.](image)

<table>
<thead>
<tr>
<th></th>
<th>Ring-$^{14}$C-molinate</th>
<th>Ethyl-$^{14}$C-molinate</th>
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</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Molinate</td>
<td>▼</td>
<td>▲</td>
</tr>
<tr>
<td>Soil-bound residues</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>$^{14}$CO$_2$</td>
<td>□</td>
<td>□</td>
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</table>
activity in the trap solution was also attributed only to $^{14}$CO$_2$ and none to $^{14}$C-ethyl-mercapthane.

$^{14}$CO$_2$ was largely evolved together with rapid decrease of molinate in the soils under upland conditions but very little under flooded conditions. The radioactivity of so-called "soil-bound residue" was not large, especially under flooded conditions.

Radioactivity in the methanol extracts decreased a little more rapidly when ethyl-$^{14}$C-molinate was treated in Anjo soil than when ring-$^{14}$C-molinate was treated, and the "soil-bound radioactivity" and $^{14}$CO$_2$ evolution were a little larger in the former treatment. These differences in the $^{14}$CO$_2$ evolution and the bound residue accumulation, however, were very small, much smaller than expected, between the treatments of the 2 kinds of labelled molinate. These indicated that both ethyl moiety and azepine ring of molinate were rapidly degraded to $^{14}$CO$_2$ at approximately the same rate under either water condition.

The total recovery of added radioactivity was more than 85% during the experimental periods in any case.

2. Analysis of Degradation Products

Figure 2 shows thin layer chromatograms of each organic fraction. It was confirmed by repeated co-chromatography that spots 1 and 4 corresponded to molinate-sulfoxide and spot 7 to molinate-sulfone. 4-OH-molinate (spot 2) and 2-oxo-molinate (spot 6) were identified by GC and GC-MS by comparison with standard compounds. Spot 3 was identical with 4-oxo-molinate, because its reduction product by NaBH$_4$ in NaOH solution (pH 10) was 4-OH-molinate. Fifty to seventy percent of the radioactivity of spot 10 was revealed to be attributable to HMI when the radioactive compounds on spot 10 were extracted and again subjected to co-chromatography with solvents D or F. Spot 14 was identical with molinate-alcohol by repeated tlc. This compound was not confirmed by GC or GC-MS because its amount was too small. In acidic ether fraction, only spot 18 was detected in a relatively large amount, and was identified with molinate-acid by repeated tlc and GC analysis after methylation with diazomethane. Ethyl-$^{14}$C-molinate did not yield spot 12 or 13 in any case. Spots 4 and 7 are considered to be artifacts during the tlc process from the behavior of molinate on tlc and also because these compounds in soil should be distributed to the basic ether fraction.

2-Oxo-hexamethylene imine (2-oxo-HMI, caprolactum), 4-OH-HMI, N-acetyl HMI, methyl ester of HMI-thiocarbamate, methyl ester of molinate-acid, ring opened molinate (S-ethyl-N-(5-carboxypentyl) thiocarbamate)

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**Fig. 2 Thin layer chromatograms of organic extracts.**

Solvent systems: same as Table 2.

1: molinate-sulfoxide, 2: 4-OH-molinate, 3: 4-oxo-molinate, 4: same as No. 1 (artifact from No. 8), 6: 2-oxo-molinate, 7: molinate-sulfone (artifact from No. 8), 8: molinate, 10: including HMI, 14: molinate-alcohol, 18: molinate-acid, 5, 9, 11, 12, 13, 15, 16, 17, 19, 20: unknown.

○: products from ring-$^{14}$C-molinate, ●: products from ethyl-$^{14}$C-molinate.
were not detected in comparison with authentic compounds.

3. Timecourse of Major Degradation Products

Degradation products did not greatly vary in the three soils under the two conditions. Under upland conditions, the amount of 4-OH-molinate in Nagano and Tochigi soils reached the maximum on the 10th day of incubation, and 4-oxo-molinate on the 20th day as shown in Fig. 3. This suggests that 4-OH-molinate produced was further oxidized to yield 4-oxo-molinate. In Anjo soil, the amount of both chemicals reached the maximum on the 10th day and decreased rapidly thereafter, corresponding to the rapid degradation of molinate. The amounts of most intermediates appeared at their maximum quantities between 10 and 20 days, and then decreased rapidly in the three soils.

Under flooded conditions, the amounts of major intermediates, especially 4-OH-molinate and molinate-acid, increased up to 40-80 days during the incubation to form larger amounts than under upland conditions in Anjo and Nagano soils, although the rates of production were slow. In Tochigi soil, however, molinate was degraded very slowly and the amount of degradation products was very small.

On the whole, ring oxidized products were detected in larger amounts under upland conditions; on the other hand ethyl moiety oxidized products were greater under flooded conditions.

As shown in Table 2, molinate sulfoxide was produced in a large amount just after molinate was added, and then decreased. HMI did not seem to accumulate in any case. Other minor products not shown in Fig. 3 or Table 2 were detected temporarily but in very small amounts (less than 0.1% of added radioactivity).

$^{14}$C-ethane sulfonate was measured in Anjo soil which was applied with ethyl-$^{14}$C-molinate. Thin layer chromatograms of the ion-exchanged samples showed 4 radioactive spots with $R_f$ values at 0.00, 0.40, 0.63, and 0.87. The spot of $R_f$ 0.63 was consistent with authentic ethane sulfonate. $^{14}$C-ethane sulfonate was detected in small amounts and disappeared rapidly (Table 2).

4. Degradation in the Sterile Soils

In the sterile soils, the decrease of molinate

![Fig. 3 Changes in amounts of degradation products in different soils.](image-url)
Table 2  Timecourses of molinate sulfoxide, HMI and ethane sulfonate in different soils and conditions (% of applied radioactivity).

<table>
<thead>
<tr>
<th>Degradation products</th>
<th>Upland conditions</th>
<th>Flooded conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  10 20 40 80</td>
<td>0  10 20 40 80</td>
</tr>
<tr>
<td>Anjo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfoxide</td>
<td>1.8 1.0 0.3 0.9 0.1</td>
<td>1.2 0.1 0.1 0.1 0.1</td>
</tr>
<tr>
<td>HMI</td>
<td>0 2.0 1.6 1.0 0.1</td>
<td>0 1.3 0.2 0.3 0.2</td>
</tr>
<tr>
<td>Ethane sulfonate†</td>
<td>0 0.6 0.4 0.1 0</td>
<td>0 0.8 1.2 0.3 0</td>
</tr>
<tr>
<td>Nagano</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfoxide</td>
<td>2.1 2.3 0.6 0.8 0.3</td>
<td>2.4 0.1 0.8 0.1 0.2</td>
</tr>
<tr>
<td>HMI</td>
<td>0.5 0.4 0.1 0.1 0.1</td>
<td>tr. 0.4 1.2 0.1 0.7</td>
</tr>
<tr>
<td>Tochigi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfoxide</td>
<td>3.7 1.6 1.7 0.2 0.1</td>
<td>2.0 tr. 0.6 0.1 0.1</td>
</tr>
<tr>
<td>HMI</td>
<td>2.6 1.6 0.2 0.1 0.1</td>
<td>tr. 1.7 0.6 0.3 0.1</td>
</tr>
</tbody>
</table>

tr.: less than 0.1%.
† Only Anjo soil was applied with ethyl-14C-molinate as well as ring-14C-molinate.

was very slow compared with unsterile soils, and the differences in the decreasing rate among the soils and the conditions were not remarkable (Fig. 4).

DISCUSSION

1. Relationship between Molinate Degradation and Soil Properties and Conditions

The degradation of molinate was more rapid under upland conditions than under flooded conditions (Fig. 1) and was suppressed by the sterilization of soil (Fig. 4). It is presumed that the degradation may be caused mainly by aerobic microbes. This result was the same as in cases of the degradation of molinate reported by Thomas et al.7) and another thiocarbamate herbicide benthiocarb.10) Under each soil condition, the degradation rates varied largely among the soils. Under upland conditions, the half life of molinate was 8, 20 and 25 days in Anjo, Tochigi, and Nagano soil, respectively. Under flooded conditions, the half life was 40 and 70 days in Nagano and Anjo soil, respectively, and 74% of the molinate still remained on the 80th day in Tochigi soil (Fig. 1).

\( Eh \) value ranged between +600—670 mV during the incubation period in each soil under upland conditions. Under flooded conditions, the value was +354, +594, +434 mV in Anjo, Nagano, and Tochigi soil, respectively, and 74% of the molinate still remained on the 80th day in Tochigi soil (Fig. 1).

![Fig. 4 Changes in amounts of molinate in sterile and unsterile soils.](image-url)
incubation, respectively. After these days, the $Eh$ values ranged between $-200$ and $-260$ mV. Tochigi soil was in the most reductive state and resulted in the slowest degradation of molinate. Such reductive conditions may be unfavorable for the growth and/or activity of molinate-degrading microbes. The oxygen-addition reactions in molinate degradation (Fig. 5) may hardly proceed under such conditions. In Nagano soil under flooded conditions, the degradation rates might be greater than in the other soils probably because of its comparatively high $Eh$ value. In a similar experiment,\textsuperscript{10} the degradation rates of benthiocarb were almost the same among these three soils, but those of molinate varied.

2. Degradation Pathways of Molinate in Soil

From degradation products and their change in amounts with time, three main routes of molinate degradation were proposed as shown in Fig. 5. In the first route, the sulfur atom of molinate is oxidized to yield sulfoxide and sulfone, and the latter may be hydrolyzed to HMI. This reaction was recognized to proceed even through the chromatography process as well as in soil, so this reaction may occur chemically as in the case of benthio-carb.\textsuperscript{11,12}

The second route is oxidation of 2 and 4 position carbons of the azepine ring. 4-OH- and 4-oxo-molinate were detected. 2-oxo-molinate which had not been recognized by Thomas \textit{et al.}\textsuperscript{7} in soil, was also detected though 2-OH-molinate was not. Under upland conditions, a large amount of $^{14}$CO$_2$ was evolved from ring-2-$^{14}$C with decrease in ring-oxidized intermediates, so azepine ring fission may rapidly occur after ring oxidation to yield CO$_2$. Spots 12 and 13 in the basic ether fraction were not detected when ethyl-$^{14}$C-molinate was added. These spots were therefore attributed to the products of ring oxidation with liberation of the ethyl moiety, probably kinds of oxidized HMI based on $Rf$ values and fractionation behavior. Each of these spots appeared temporarily and their radioactivities were less than 0.1\% of added $^{14}$C. Therefore, it is presumed that even when the ethyl moiety is separated from the ring-oxidized molinate, the ring is opened rapidly to yield hydrophilic compounds and further CO$_2$.

The third route is oxidation of ethyl moiety. The ethyl moiety is oxidized to yield molinate-alcohol and further molinate-acid. Molinate-alcohol was newly detected but only in a small amount.

It was suggested that the second route might be the main degradation pathway under upland conditions, while the third route might mainly occur under flooded conditions. This suggested that many kinds of microbes might degrade molinate in soil and molinate-degrading microflora might be different for upland and flooded conditions.

Kaufman\textsuperscript{15} proposed that thiocarbamate herbicides might be degraded mainly by hydrolysis of N-CO-S bonds in soil. However, from the results of this experiment, it was
elucidated that the main degradation reaction of molinate was oxidation of alkyl carbons under the two moisture conditions.

Thomas et al.\(^7\) also proposed molinate degradation pathways in soil as follows: molinate → HMI → N-acetyl HMI and molinate → HMI-thiocarbamate → methyl ester of HMI-thiocarbamate. These degradation products, however, were not detected in comparison with authentic compounds in this study. Golovleva et al.\(^7\) recognized 2-OH-molinate and HMI-thiocarbamate as the degradation products by Mycococcus sp. and Skryabin et al.\(^2\) also recognized 3-OH-, ring 3 : 4 double bonded-, ring-di oxo- and ring-hydroxy-oxo-derivatives of molinate and oxidized HMI-thiocarbamates as the degradation products by Micrococcus sp. and Bacillus sp. In this study, however, all products in the acidic ether fraction derived from ring-\(^{14}\)C-molinate were the same as those from ethyl-\(^{14}\)C-molinate. Therefore, it can be said that free acid of HMI-thiocarbamate or ring-oxidized HMI-thiocarbamate was not found in the soils in this study. The other products that Golovleva and Skryabin recognized did not appear in sufficient amounts to be identified in this study.

Under upland conditions, time lags were recognized between two steps of ring oxidation, in Nagano and Tochigi soils (Fig. 3). The maximum of the amount of 4-OH-molinate appeared approximately at the 10th day, and 4-oxo-molinate at the 20th day and then decreased (Fig. 3). This probably means that, in this route of molinate degradation, more than one kind of microbe work together to degrade molinate to CO\(_2\), and the situation may be similar in other routes. Under flooded conditions, the microbial activities in the oxidation of 4-OH-molinate to 4-oxo-molinate and 4-oxo-molinate to CO\(_2\) may be lower than the activities in the oxidation of molinate to 4-OH-molinate.

3. Soil-bound Residue
It is known that amino derivatives produced from some substituted diphenyl ether herbicides\(^{16-19}\) and amide herbicides\(^{20}\) in soil are bound on soil particles to persist for a long time against further degradation. Similarly, in the case of molinate, the degradation products HMI and its hydroxy or oxo derivatives of imines were though to be persistent in soil as bound residues. Radioactivity of soil-bound residues, however, was relatively little even when ring-\(^{14}\)C-molinate was added (Fig. 1), so it is elucidated that no degradation products of molinate will persist in a large amount in soil.

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REFERENCES

要約
除草剤モリネットの土壌中における分解
今井康史、鎌塚昭三
モリネットの土壌中における分解経路と土壌条件による分解の相違について、室内実験で3種類の水田土壌と、アゼビン環ないしS-エチル基をラベルしたモリネットおよび無標識化合物を用い研究した。畑地状態と洪水状態で分解を比較したところ、土壌間差が顕著であるが、いずれの土壌でも畑地状態の方が分解速度および14CO2発生量が大きかった。また滅菌土壌ではモリネットの消失速度は著しく小さかった。分解物として、これまでに報告のあるスルホキシド、アゼビン環-4-OH体および4-オキソ体、S-カルボキシルメチル体およびヘキサメチレンイミンが認められ、今回新たにアゼビン環-2-オキソ体、S-2-OH-エチルおよびエタノールホン酸が認められた。分解経路として、1) イオウ原子の酸化、2) アゼビン環の酸化、3) S-エチル基の酸化、4) 加水分解によりヘキサメチレンイミンないしエタノールホン酸を生ずる経路が想定され、畑地状態では2) か、洪水状態では3) が主要分解経路であると考えられた。土壌の性質とモリネットの分解の関係についても考察した。