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Degradation of the Herbicide, Alachlor, by Soil Microorganisms

Ⅲ. Degradation under an Upland Soil Condition

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제초제 Alachlor의 토양미생물에 의한 분해

제 3 보. 밭토양 조건에서의 분해

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초　록

제초제 Alachor가 밭토양 조건하에서 80일간 배양될때 다음과같은 4개의 주요분해 산물을 생성하였다. 즉 8-ethyl-2-hydroxy-N-(methoxymethyl)-1,2,3,4-tetrahydroguinoline (m/z 221). N-hydroxyacetyl-2,3-dihydro-7-ethylindole (m/z 205), 2-Hydroxy-2′, 6′,-diethyl-N-(methoxymethyl) acetanilide (m/z 251) 그리고 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-beznoxazepin-2(3H)-one (m/z 249)이었다. 이들 분해산물은 제 1보에서 사용한 담수 논 토양조건하에서 얻었던 분해산물들과 약간 다르다는 것을 알게 되었다. 그리고 Alachlor의 가능한 분해 경로를 제시하였다.

Introduction

In the previous investigation[1], Alachlor, 2-chloro-2′,6′-diethyl-N-(methoxymethyl) acetanilide, was shown to produce 1-formyl-,3-dihydro-7-ethylindole, 2,6-diethylaniline, 2,6-diethyl-N-(methoxymethyl) acetanilide, 2-hydroxy-2′,6′-diethyl-N-(methoxymethyl) acetanilide, and three unidentifiable compounds as its degradation products, when incubated under the flooded paddy soil condition. Of all these products, 2,6-diethyl-N-(methoxymethyl) acetanilide and 2-hydroxy-2′,6′-diethyl-N-(methoxymethyl) acetanilide turned out to be the major degradation products and were synthesized for the purpose of examining their phytotoxicity, using rice, radish, and mungbean seeds

at their germinating stage[2].

In the present investigation, the degradation products of Alachlor obtained under an upland soil condition were elucidated, since the products were a little different from those obtained under the flooded paddy soil condition.

Materials and Methods

The soils used

The physico-chemical properties of the sample soils are presented in Table 1.

Incubation of the soils treated with Alachlor

100g of each soil was put in 500-ml erlenmeyer flasks and 100 ml of distilled water added. Alachlor dissolved in a small volume of acetone was added to the flasks to give a final concentration of 500 ppm and the soil and Alachlor were mixed well. The soil moisture was adjusted to be 60% of the maximum water-holding capacity of each soil by adding distilled water once a week. The Alachlor-treated soils were incubated at 28°C for 80days. For the controls, 100 g of each soil was autoclaved at 121°C for 30min, the same procedure being repeated two more times. After sterilizing the soils, 100ml of sterile distilled water was added to them and the wet soils were incubated in the same way as the above.

Extraction of the soils

After the given periods of incubation time, the soils were extracted with 50 ml of MeOH by shaking for 30 min at 180 rpm on a rotary shaker and by centrifuging at 4,000 rpm for 5 min. The same extraction procedure was repeated 5 times. The extracts combined after filtration were concentrated to almost dryness to remove MeOH. The water residues were extracted with $CHCl_3$ three times and the $CHCl_3$ extracts were concentrated to small amounts by air bubbling.

Gas-liquid chromatography

The gas-liquid chromatograms were obtained with an instrument of Shimadzu, gas chromatograph GC-4C(PTF) equipped with a flame ionization detector. The column was a pyrex glass of 6 mm(OD)×8 ft packed with 5% silicone rubber SE-30 on 60-80 mesh Shimalite W(AW-DMCS). The operating parameters are as follows: column temp., 170°C; injection port temp., 220°C; detector temp., 250°C. Helium carrier flow, 50ml/min.; air, 0.8kg/cm^2; hydrogen, 0.7kg/cm^2. For better resolution, a Hewlett-Packard HP 5840 A GC equipped with a flame ionization detector was also used. The column was an OV-101 fused silica capilliary

Table 1. Physico-chemical properties of the soils used

Sample soils	Sand(%)	Silt(%)	Clay(%)	Textural class	pH (1:1) H_2O	pH (1:1) 1N KCl	pH (1:1) 0.01N $CaCl_2$	C(%)	O.M.(%)
A	81.3	13.7	5.0	Loamy sand	5.7	5.1	5.0	2.2	3.79
B	67.9	25.7	6.4	Sandy loam	4.9	3.6	4.1	4.11	7.09

	Available P_2O_5(ppm)	T-N(%)	Exchangeable cation (me/100g) K	Na	Ca	Mg	C.E.C. (me/100g)
A	80	0.07	0.32	—	2.6	1.0	4.4
B	100	0.08	0.38	—	3.7	1.2	7.5

column(0.2 mm i.d.×12 m). Helium carrier flow was 20cm/sec. The column temp. was programmed from 160°C (3min.) to 240°C at a rate of 5°C/min.

Mass spectrometry

A Hewlett-Packard HP 5985 BGC/MS system was used fnr the GC-MS. For the direct inlet probe of the purified sample for further information. a Kratos MS 25 was also used. The source temp. was 200°C and the electron ionization potential was 70 eV in both cases.

IR spectrometry

The IR spectrum was obtained using a Perkin-Elmer model 298. Chloroform was used as the dissolving solvent for the sample.

Thin-layer chromatography

As the preparative plates, Kieselgel GF 254 (Typ 60) (Art. 7730, Merck) was coated on 20×20cm glass plates and used after activation at 110°C for 3 hr. The precoated analytical plates of silica gel HF-254 with a fluorescent indicator were used for comparison of the Rf's the test compounds. The developing solvents were the same as those used in the previous papers[1,2].

Results and Discussion

Formation of the degradation products

Fig. 1 shows the gas-liquid chromatogram of the degradation products of Alachlor which was incubated under an upland soil condition for 80 days (Shimadzu GC-4C). As can be seen in this spectrum, the controls did not show any products except for the solvent peak, whereas both soil A and B treated with 300ppm of Alachlor formed a few degradation products, in addition to the biggest peak of the intact Alachlor.

Elucidation of the degradation products

Since the degradation products of Alachlor

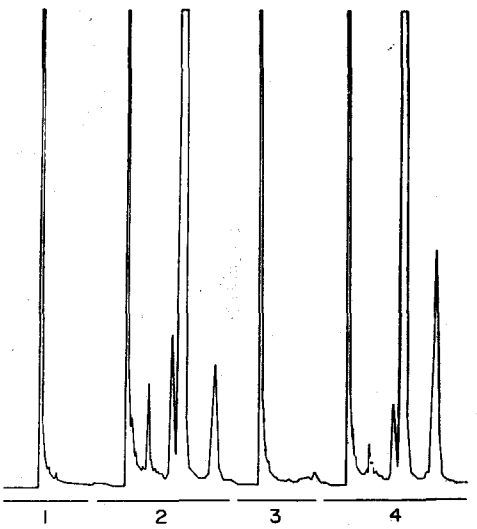

Fig. 1. Gas-liquid chromatogram showing the microbial degradation of Alachlor which was incubated under an upland soil condition for 80 days.
1: Control (extract from soil A without Alachlor)
2: Extract from Alachlor(300 ppm)-treated soil A
3: Control (extract from soil B wit hout Alachlor)
4: Extract from Alachlor (300 ppm) treated soil B

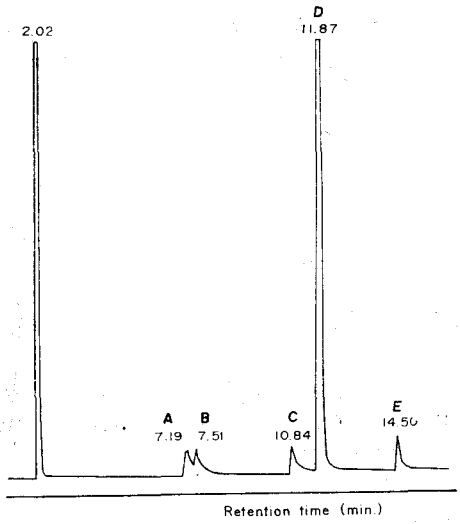

Fig. 2. Gas-liquid chromatogram obtained with a capillary column of the extract from soil B which was treated with 300 ppm-Alachlor and incubated under an upland soil condition for 80 days.

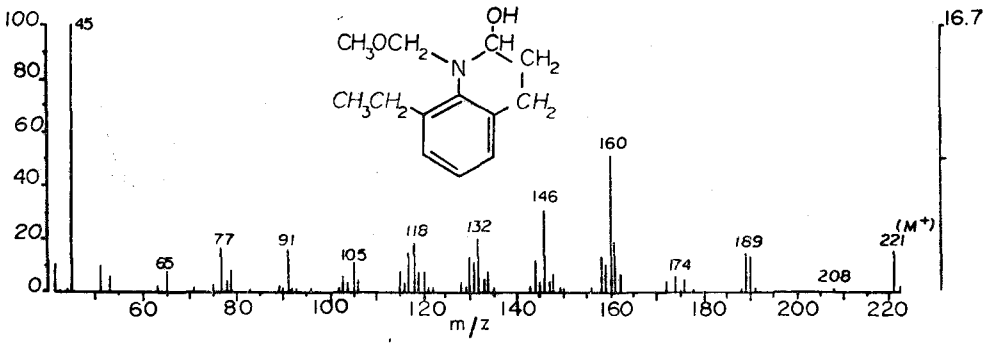

Fig. 3. Mass spectrum of product A, 8-ethyl-2-hydroxy-N-methoxymethyl-1,2,3,4-tetrahydroquinoline.

obtained from soil A and B were all alike, the extract from soil B was used for the GC-MS. Fig. 2 shows the chromatogram obtained with a capillary column as described in materials and methods. Each peak was elucidated with a mass spectrometer which was hooked up with the gas liquid chromatograph.

Product A (Retention time: 7.19) : Fig. 3 shows the mass spectrum and proposed che mical structure of the product A. In this spec trum, the molecular ion appears at m/z 221 with the base peak at m/z 45 which corresponds to $(CH_3OCH_2)^+$. The fragment peaks at m/z 190 and 176 are thought to fit M-CH_3O and M-CH_3OCH_2, respectively. Based on these observations, this product was termed 8-ethyl -2-hydroxy-N-methoxymethyl-1,2, 3,4-tetrahydroquinoline.

Product B (retention time: 7.51) : Fig. 4 shows the mass spectrum and proposed chemical structure of the product B. As can be seen in this spectrum, the molecular ion appears at m/z 205. The major fragment peaks at m/z 188 and 174 are believed to correspond to M-OH and M-CH_2OH, respectively. Based on this fragmentation pattern, the entity of this product is believed to be N-hydroxyacetyl-2,3-dihydro-7-ethylindole. Tiedje and Hagedorn[3] reported N-chloroacetyl-2,3-dihydro-7-ethylindole as one of the degradation products of Alachlor by a soil fungus, *Chaetomium globosum*. The same product was also found by Mc Gahen and Tiedje[4] in the degradation of Antor herbicide which is an analogous acylanilide of Alachlor. The N-hydroxyacetyl derivative which was found in the present investigation is believed to be readily formed by dechlorination followed by subsequent hydroxylation.

Product C (retention: 10.84) : The mass spectrum and proposed chemical structure of prod-

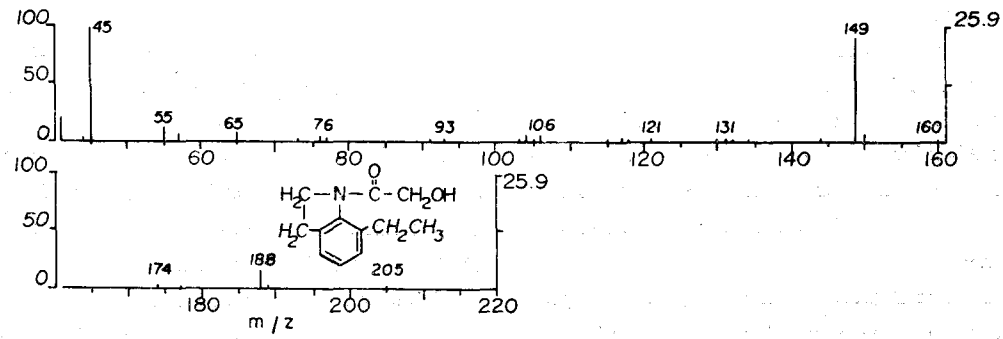

Fig. 4. Mass spectrum of product B, N-hydroxyacetyl-2,3-dihydro-7-ethylindole.

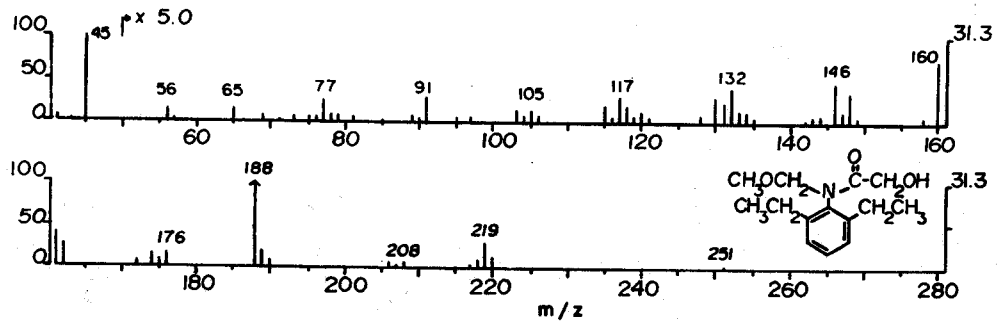

Fig. 5. Mass spectrum of product C, 2-hydroxy-2′,6′-diethyl-N-(methoxymethyl) acetanilide.

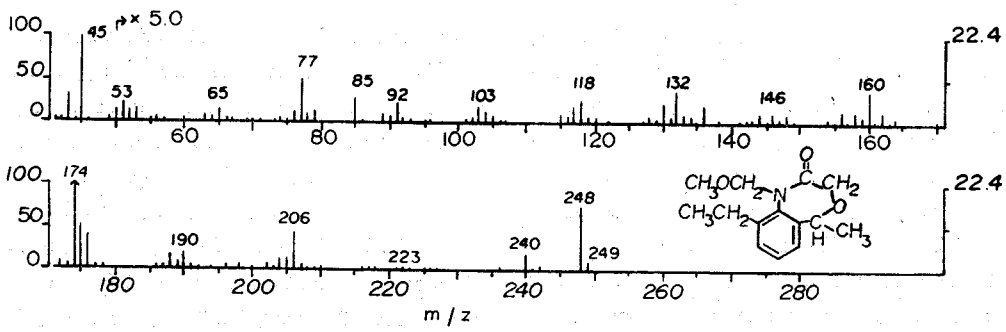

Fig. 6. Mass spectrum of product E, 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2(3H)-one (GC-MS).

uct C are shown in Fig. 5. In this spectrum, the molecular ion occurs at m/z 251 as a weak peak. The major fragment peaks at m/z 220, 219, and 206 correspond to M-OCH_3, M-CH_3OH and M-CH_3OCH_2-respectively. The base peak appears at m/z 45 which corresponds to$(CH_3OCH_2)^{+\cdot}$. The chemical structure which fits these observations is believed to be 2-hydroxy-2′,6′-diethyl-N-(methoxymethyl) acetanilide. This product was also formed under the flooded paddy soil condition used in the previous research.[1] The same evidence of dechlorination and subsequent hydroxylation of the N-chloroacetyl moiety in the structure of Alachlor was also observed in the microbial metabolism of Alachlor and its related acylanilide herbicides, Antor and Metolachlor.[3,4]

Product D (retention time: 11.87) : This product has the molecular ion at m/z 269, which corresponds to the intact Alachlor.

Product E (retention time: 14.50) : Fig. 6 shows the mass spectrum of the product E and its proposed chemical structure. As can be seen in this spectrum, the molecular ion occurs at m/z 249 and the prominent fragment ion at m/z 248 seems to correspond to the M-H peak. The major fragment at m/z 206 is conistent with the possible M-CH_2CHO peak. And besides, the fragments at m/z 190 and 160 are thought to correspond to the M-CH_3CONH_2 and M-CH_3CONH_2, CH_2O, respectively. The base peak at m/z 45 is believed to be the fragment ion, $(CH_3OCH_2)^{+\cdot}$. The same product was also formed under the flooded paddy soil condition described in the previous paper.[1] However, its chemical structure could not be elucidated at that time. Based on the degradation pattern of Butachlor which is an analogous acylanilide herbicide of Alachlor and was described in the previous paper[1], the formation of this product could be easily verified. For further con-

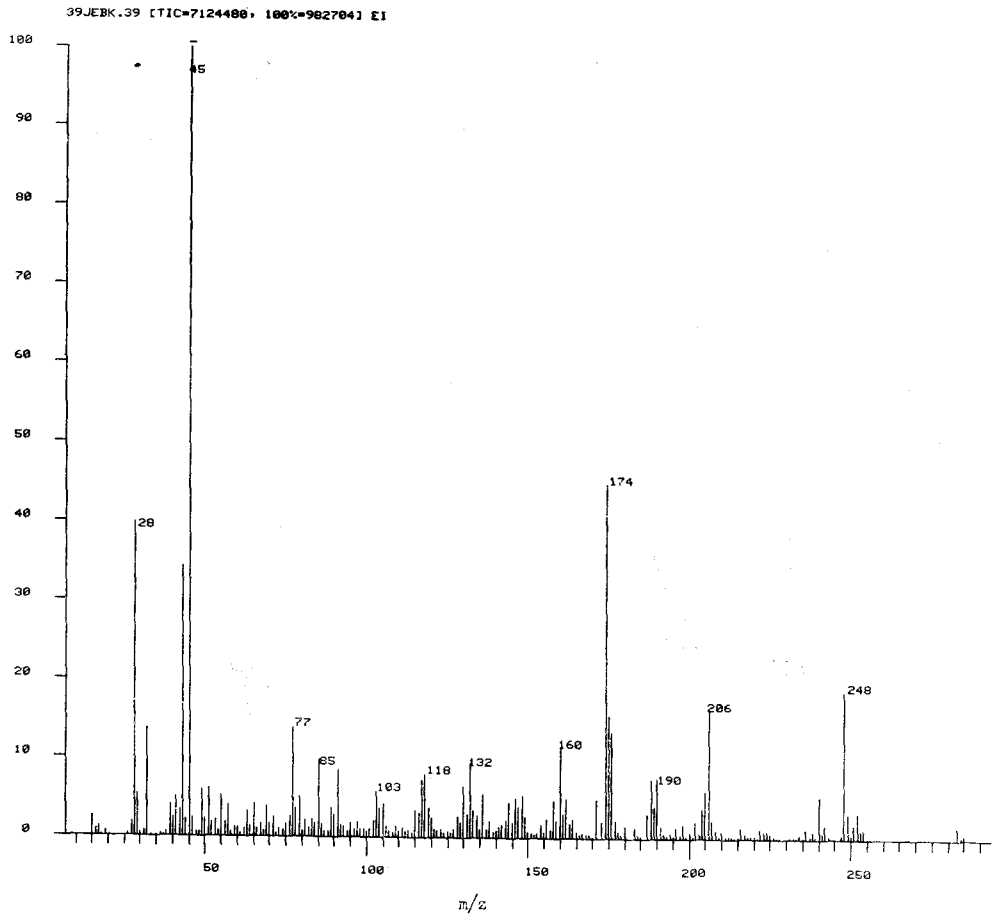

Fig. 7. Mass spectrum of product E, 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2(3H)-one (direct probe).

firmation, the mass spectrum of this product was taken again by direct probe as seen in Fig. 7. The fragmentation pattern in this spectrum is identical with that of the above GCMS. The small peaks beyond m/z 249 are believed to be due to impurities. Mc Gahen and Tiedje[4] proposed the oxoquinoline formation in the metabolism of Antor herbicide and Metolachlor which are the analogous acylanilide herbicides of Alachlor. According to their conclusion, the product E which has the molecular ion of m/z 249 could be 8-ethyl-3-hydroxy-N-(methoxymethyl)-4-methyl-2-oxo-1,2,3,4-tetrahydroquinoline, as can be seen in Fig 8 which also shows another plausible structure. For its confirmation, the IR spectrum of the product E was

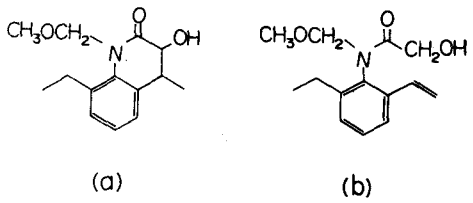

Fig. 8. The other plausible chemical structures having m/z 249.
(a) 8-Ethyl-3-hydroxy-N-(methoxymethyl)-4-methyl-2-oxo-1,2,3,4-tetrahydroquinoline
(b) 2-Hydroxy-N-(2′-ethyl-6′-vinylphenyl)-N-(methoxymethyl) acetamide

obtained as shown in Fig. 9. In this spectrum, there is no characteristic absorption band cor-

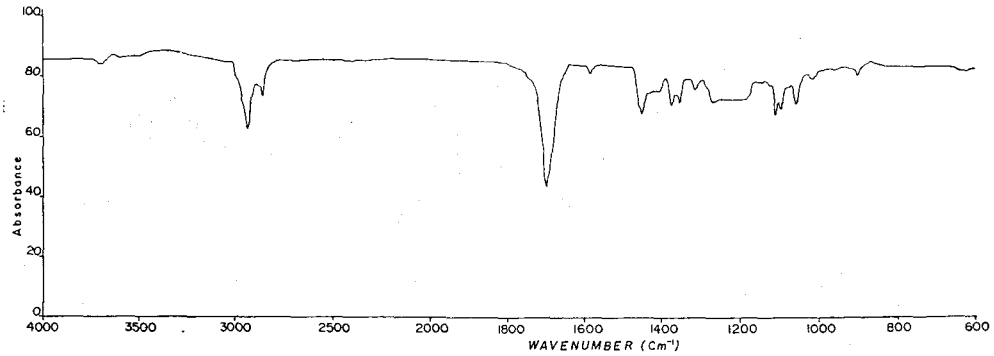

Fig. 9. IR spectrum of product E.

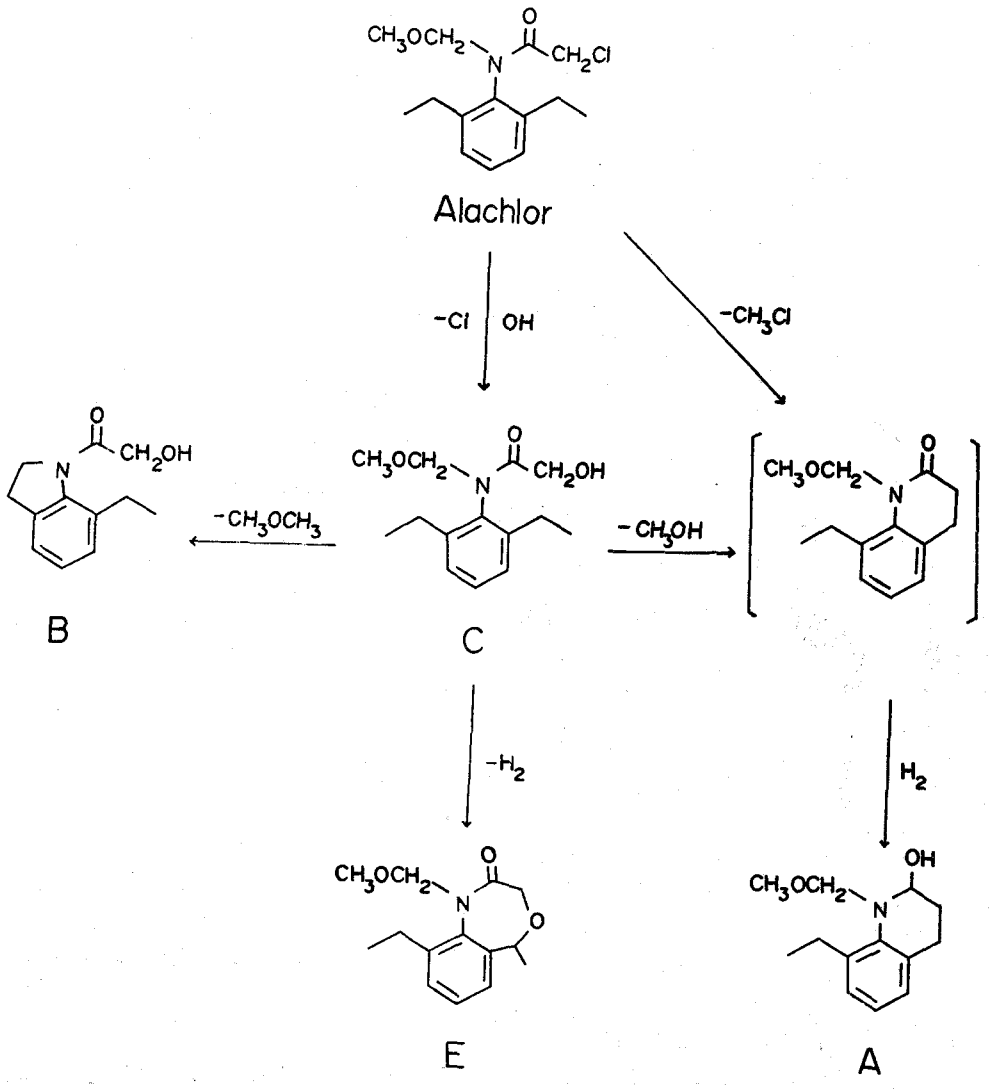

Fig. 10. Plausible pathways for the degradation of Alachlor under an upland soil condition.

responding to the OH stretching vibration. Accordingly, neither a nor b in Fig. 8 can be the suitable structure for this product. Instead, the proposed structure was named 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2(3 H)-one.

Comparison of the degradation patterns of Alachlor under the different soil conditions

The plausible pathways of Alachlor degradation under an upland soil condition are shown in Fig. 10. Product A, 8-ethyl-2-hydroxy-N-(methoxymethyl)-1, 2, 3, 4-tetrahydroquinoline and product B, N-hydroxyacetyl-2,3-dihydro-7-ethylindole could not be detected under the flooded paddy soil condition[1]. Meanwhile, 2,6-diethyl-N-(methoxymethyl) acetanilide which was one of the major degradation products in the flooded paddy soil[1] and turned out to be ineffective in the phytotoxicity test[2] was not produced under the present upland condition. Product C which turned out to be 2-hydroxy-2′,6′-diethyl-N-(methoxymethyl) acetanilide was formed as one of the major products both under the flooded paddy and the upland soil conditions. In the previous paper[2], the phytotoxicity of this product was also evaluated. Product E which is named 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2(3H)-one was also formed in the flooded paddy soil. In the previous investigation[1], however, this product could not be elucidated. The other minor products which were formed under the flooded paddy condition were not noticeable in the present investigation.

Abstract

Alachlor, 2-chloro-2′,6′-diethyl-N-(methoxymethyl) acetanilide produced four major degradation products, when incubated under an upland soil condition for 80 days. They include 8-ethyl-2-hydroxy-N-(methoxymethyl)-1, 2, 3,4-tetrahydroquinoline (m/z 221), N-hydroxyacetyl-2,3-dihydro-7-ethylindole (m/z 205), 2-hydroxy-2′,6′-diethyl-N-(methoxymethyl) acetanilide (m/z 251), and 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2 (3H)-one (m/z 249). The products turned out to be a little different from those obtained under the flooded paddy soil condition used in the previous paper. The plausible pathways for the degradation were proposed.

Acknowledgment

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