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Degradation of the Herbicide, Butachlor, in Soil

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除草劑 Butachlor의 土壤中 分解

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抄　錄

二種의 土壤을 가지고 논과 유사한 狀態로 만들어 butachlor를 一定期間 培養時 모든 경우 主分解産物로 2, 6-diethyl-N-(butoxymethyl) acetanilide와 新分解産物 C를, 그리고 경우에 따라서는 比較的 少量의 新分解産物 8-ethyl-2-hydroxy-N-(butoxymethyl) 3, 4-dihydroguinoline을 生成하였다. 土壤微産物의 作用으로 推測되는 이 分解는 어느 정도 배양기간에 비례하여 進行되었다. 전번 연구의 혐기적 배양시에는 2, 6-diethyl-N-(butoxymethyl) acetanilide가 主分解産物인 반면 이번 경우에는 m/z 291의 物質이 主分解産物이었다.

그리고 Mass Spectrum의 Fragmentation Pattern에 依하여 이들 新分解産物의 化學構造를 究明하고 可能한 生成經路를 推定하였다.

Introduction

Along with alachlor, butachlor, 2-chloro-2′, 6′-diethyl-N-(butoxymethyl) acetanilide, has been in wide use as a preemergence acetanilide herbicide in Korea. Baird[1] separated three organic soluble and five aqueous soluble metabolites in his study on butachlor degradation in the environment. Two of the organic soluble metabolites were tentatively identified as the N-dealkylated and the α-hydroxy derivatives. Chen and Wu[2] reported 2-chloro-2′, 6′-diethylacetanilide, 2-hydroxy-2′, 6′-diethylacetanilide, 2, 6-diethylaniline, N-chloroacetyl-7-ethyl-2, 3-dihydroindole, 2, 6-diethylacetanilide and N-methyl-2-chloro-2′, 6′-diethylacetanilide as the microbial metabolites of butachlor by *Mucor sufui* NTU-358. Lee[3] elucidated five metabolites of butaclor incubated in the pure culture of *Chaetomium globosum.*

Quite recently, Lee et al.[4] reported 2, 6-diethyl-N-(butoxymethyl) acetanilide, 2, 6-diethylaniline, and 2, 6-diethylacetanilide in an anaerobic degradation of uniformly ring-labeled ^{14}C-butachlor in soil. In the present investigation, butachlor was incubated in a simulated rice-paddy soil condition which was somewhat aerobic, for two, four and six months, and the degradation products obtained from the soils were elucidated in comparison with those of the previous anae-

robic incubation.

Materials and Methods

Soils used.

The physico-chemical properties of the soils used are the same as those of the previous samples.[4] The main characteristics are as the following; soil A(Cheong Ju soil). Total sand, 43.1%; Silt, 41.4%; Clay, 15.4%; Textural class, Silty loam; pH, 4.83 : organic matter, 2.45%; C.E.C., 7.7 me/100g.

Soil B(Chung Ju soil). Total sand, 30.5%, Silt, 53.1%; Clay, 16.5%; Textural class, Clay loam; pH, 6.43; Organic matter, 2.9%; C.E.C., 14.14 me/100g.

Incubation.

100g of soil A and B, each, was put in 500 m*l* Erlenmeyer flasks and 100m*l* of distilled water added to each of them. Butachlor dissolved in acetone was added to each flask to give a final concentration of 500ppm and the mixture was shaken thoroughly. The water layer above soil was kept about 0.5cm. The incubation was continued at 28°C for two, four, and six months, respectively. As the controls, each of 100g of soil A and B was sterilized by autoclaving at 121°C for 30min and 100ml of sterile distilled water was added to each flask in the same manner.

Extraction of soils after incubation.

After certain periods of incubation, water was separated by centrifugation. The soils were then extracted with four 100m*l* portions of methanol and two 100m*l* portions of acetone by shaking for 30min at 180 rpm on a shaker. The samples were centrifuged at 3,500rpm for five min in each extraction. The extracts which were collected after filtration and combined were concentrated to a small volume.

Gas-liquid chromatography.

The analyses were performed with a Gas Chromatograph GC-4C(PTF) equipped with a flame ionization detector. The column was a pyrex glass of 6mm(OD)×8ft packed with 5% Silicone GE SE-30 on 60-80 mesh Shimalte W (AW-DMCS) 201 D. Operating parameters were as follows: helium carrier flow, 50m*l*/min; air, 0.8Kg/cm²; hydrogen, 0.7Kg/cm²; injection temp., 230°C; detector temp., 240°C. All analyses were performed by temp-programming from 120 °C to 240°C at a rate of 5°C/min.

Mass spectrometry.

Mass spectra for GLC-MS and direct inlet probe were obtained by using a Finnigan 3200 Gas Chromatograph-Mass spectrometer and a Varian Mat 212 Mass Spectrometer. The electron ionization potential was 70eV and the emission current 1mA.

Infrared spectrometry.

Perkin-Elmer 599B Infrared Spectrophotometer was used for the IR spectrum. The sample dissolved in a small amount of chloroform was coated on a cell and the solvent was left to evaporate for a while to make a thin film for obtaining the spectrum.

Thin-layer chromatography.

In order to isolate and purify the degradation product C, Kieselgel CF 254(Typ 60)(Art. 7730, Merck) was coated on 20×20cm glass plates and used after activation. For identification of the products, the precoated analytical plates of silica gel HF-254 with fluorescent indicator were used. The developing solvent was a mixture of benzene-methanol(85 : 15, v/v). The products were detected under a UV lamp.

Results and Discussion

Formation of the degradation products.

The ratios of formation of the degradation products of butachlor in soil A and B with different incubation periods were calculated based on the gas-liquid chromatograms as shown in

Table 1. Comparison of the relative amounts of the degradation products of butachlor incubated for different periods under the simulated rice-paddy conditions of two soils

Soil	Incubation period (month)	Ratio of formation of the degradation products(%)*		
		A	B	C
A	2	1.1	95.9	3.0
	4	2.3	94.2	3.5
	6	20.4	49.5	30.1
B	2	10.5	71.0	18.5
	4	4.7	59.6	35.7
	6	10.9	66.7	22.4

*A+B+C=100%
A : 2, 6-Diethyl-N-(butoxymethyl) acetanilide
B : Residual butachlor
C : A new degradation product, not named

Table 1. As can be seen in Table 1, the formation of the major degradation products did not show much difference between soil A and B, irrespective of the incubation periods up to six months. Nevertheless, the maximum ratio of formation of the degradation product A was 20.4% in soil A at the incubation period of six months. In the meantime, the maximum ratio of 35.7% was formed in soil B at the incubation period of four months. On the basis of these results, it seems that there is no positive correlation in forming the degradation products with time within these periods of incubation. However, it is obvious that the longer periods of incubation caused the more degradation.

Elucidation of the degradation products.

The gas-liquid chromatogram of the degradation products of butachlor incubated for six months under a simulated rice-paddy condition of soil B is shown in Fig. 1 as an example, the rest not being presented. By means of GLC-MS, the degradation product A turned out to be 2, 6-diethyl-N-(butoxymethyl) acetanilide which was previously identified as the major degradation product in the anaerobic metabolism of butachlor in soil[4]. As the mass spectrum of the product was

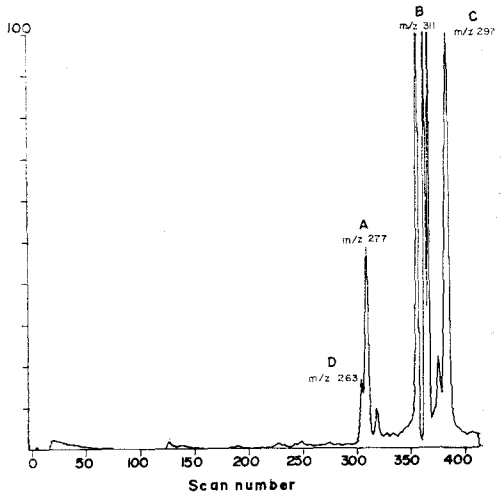

Fig. 1. Gas-liquid chromatogram of the degradation products of butachlor incubated for six months under a simulated rice-paddy condition of soil B
A : 2, 6-Diethyl-N-(butoxymethyl) acetanilide
B : Residual butachlor
C : A new product, not named
D : Another new product, 8-ethyl-2-hydroxy-N-(butoxymethyl)-3, 4-dihydroquineline

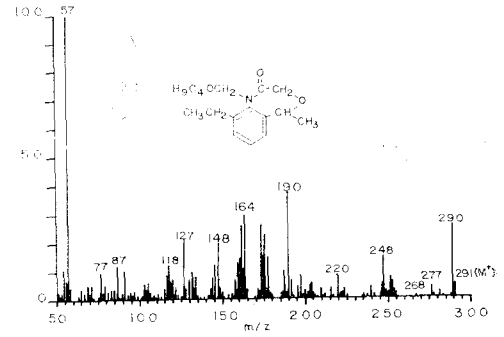

Fig. 2. Mass spectrum of the degradation product C of butachlor incubated under a simulated rice-paddy soil condition(direct inlet probe)

presented in the previous paper, it was not shown here. The product B was identified as the residual butachlor by GLC-MS. Meanwhile, the degradation product C was thought to be a new product which had not been known as yet. In order to verify its identity, the soil extract which had been used for GLC-MS was applied

on preparative TLC plates to isolate and purify the product C. Again, the mass spectrum of the product C purified by TLC was obtained. Mass spectra of the product C by GLC-MS and direct inlet probe were both completely identical. Only the mass spectrum of the product C by direct inlet probe is presented in Fig. 2. As can be seen in this mass spectrum, the molecular ion peak occurs at m/z 291 with an intense M-1 peak at m/z 290. This noticeably intense M-1 fragment peak will be due to the following resonance-stabilized ions as seen in Fig. 3. The fragment peak at m/z 277 corresponds to M-CH_2. The fragment peak at m/z 220 will be due to the loss of C_4H_9 from the fragment at m/z 277. The prominent fragment peak at m/z 190 will correspond to M-CH_2, $CH_2OC_4H_9$. In like manner, the fragment peak at m/z 248 will correspond to M-CH_2, CHO. The base peak at m/z 57 is in good agreement with the loss of C_4H_9 from the molecular ion. The fragment peak at m/z 87 corresponds to $CH_2OC_4H_9$ coming from the molecular ion. Furthermore, in the IR sepectrum of the degradation product C, the carbonyl absorption at 1690cm^{-1} was indicative of the pre-

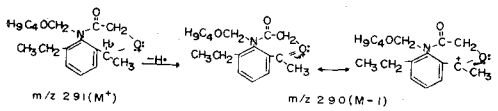

Fig. 3. A characteristic fragmentation of the molecular ion leading to the M-1 fragment peak at m/z 290, indicating the presence of the resonance-stabilized ions

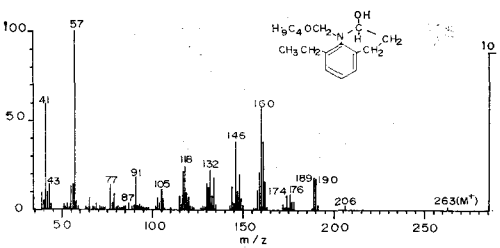

Fig. 4. Mass sepctrum of the degradation product D of butachlor incubated under a simulated rice-paddy soil condition(GLC-MS)

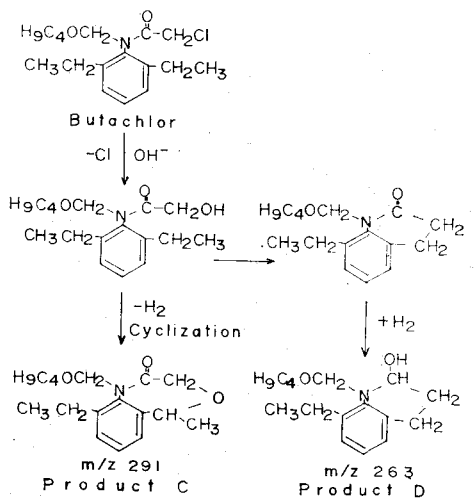

Fig. 5. Possible pathway for the formation of the new degrdation products C and D of butachlor incubated under a simulated rice-paddy condition

sence of the adjacent nitrogen atom with lone-pair electrons. However, a satisfactory spectrum was not available due to a scanty amount of the sample.

The mass spectrum of the product D which is relatively small in amount compared with the other products is presented in Fig. 4. In this sepectrum, the molecular ion appears at m/z 263. The prominent fragment peaks at m/z 206, 190, and 189 correspond to M-C_4H_9, M-C_4H_9O, and M-C_4H_9, OH, respectively. The fragment peak at m/z 160 is due to the loss of C_4H_9, OH, and CH_3CH_2 from the molecular ion. The base peak at m/z 57 indicates the presence of the intact C_5H_9 moiety in the structure. The above fragmentation pattern indicates the presence of a hydroxy group in the molecular ion. Therefore, the product D seemed to be another new one and tentatively named as 8-ethyl-2-hydroxy-N-(butoxymethyl)-3, 4-dihydroquinoline.

Finally, a possible pathway for the formation of the new products C and D via an α-hydroxy derivative is postulated as can be seen in Fig. 5. At first, the butachlor molecule can readily undergo dechlorination and subsequent hydroxylation by soil microorganisms or chemical nuc-

leophilic substitution by a hydroxide ion in soil. Baird[1] tentatively identified N-butoxymethyl-2-hydroxy-2′, 6′-diethylacetanilide as one of the three organic soluble products of butachlor incubated in flooded and non-flooded soil conitions. He believed that microbial detoxication might be the primary mechanism in butachlor degradation in soil. Chen and wu[2] reported 2-hydroxy-2′, 6′-diethylacetanilide as one of the microbial metabolites of butachlor by *Mucor sufui* NTU-358. Similar dechlorination followed by subsequent hydroxylation in the analogous herbicides, alachlor, antor, and metolachlor was postulated.[5,6] The formation of these two products C and D from this α-hydroxy derivative through dehydrogenation followed by cyclization for the formation of the product C and through cyclization followed by hydrogenation possibly by means of a certain dehydrogenase[7] for the formation of the product D is noteworthy, even if few examples are available in the literature at the moment. McGahen and Tiedje reported similar ring formations in antor[6] and metolachlor by the soil fungus, *Chaetomium globosum*, but the fragmentation patterns of the mass spectra which they have presented are quite different[6]. Additional evidence for the ring formations was observed in a previous study on degradation of butachlor by a soil fungus, *Chaetomium globosum* by Lee[3].

Interestingly enough, an outstanding difference was made between the anaerobic and the simulated rice-paddy conditions. That is, in a previous study on anaerobic metabolism of butachlor in soil, 3, 6-diethyl-N-(butoxy-methyl) acetanilide was identified as the major product, whereas in the present study dealing with a simulated rice-paddy condition which is rather aerobic, the product C was identified as the major product with 2, 6-diethyl-N-(butoxymethyl) acetanilide as the second-most product and a minor new product D was formed in some cases.

Abstract

Butachlor incubated for certain periods of time under the simulated, rather aerobic, rice-paddy conditions of two different soils exhibited two major degradation products, 2, 6-diethyl-N-(butoxymethyl) acetanilide and a new product C in all cases, and a relativly small amount of another new product D named tentatively as 8-ethyl-2-hydroxy-N-(butoxymethyl)-3, 4-dihydroquinoline in some cases. The supposedly microbial degradation seemed to proceed with incubation periods to some extent. An anaerobic incubation in the previous investigation showed 2, 6-diethyl-N-(butoxymethyl) acetanilide as the major product, whereas the new product C with m/z 291 turned out to be the major one in the present condition. Structural elucidation of the products was done based on the fragmentation patterns of the given mass spectra and a possible pathway for their formation was postulated.

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