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Clay-Catalyzed Nitration of a Carbamate Fungicide Diethofencarb

RIKA KODAKA,* TERUMI SUGANO, TOSHIYUKI KATAGI, AND
YOSHIYUKI TAKIMOTO

Sumitomo Chemical Company, Ltd., Environmental Health Science Laboratory, 2-1,
Takatsukasa 4-chome, Takarazuka 665-8555, Japan

The unique nitration of the carbamate fungicide diethofencarb (Powmyl, isopropyl 3,4-diethoxycarbanilate) was examined in 14 Japanese soils and three types of clays under the aerobic conditions using the ^{14}C-labeled compound. Nitration at the 6-position of the 3,4-diethoxyphenyl ring was a clay-catalyzed reaction and extremely enhanced under the dry conditions. Kinetic and product analysis on nitration of nine ^{14}C-labeled carbamate analogues in the kaolinite thin layer showed the nitration proceeding electrophilically. Requirement of molecular oxygen and retardation of nitration by radical scavengers and spin-trap reagents together with semiempirical AM1 molecular orbital calculations strongly suggested contribution of a radical mechanism, and these different speculations on the reaction mechanism might originate from the heterogeneous reaction environment on clay.

KEYWORDS: **Nitration; clay mineral; abiotic transformation**

INTRODUCTION

When applied to plants, the liquid of a pesticide formulation intercepted by fruits, foliage, or stem exhibits its biological activity and the other portion passes through to a ground surface. On the soil surface, a pesticide usually undergoes various transformations such as sunlight photodegradation, abiotic hydrolysis, and metabolic degradation by soil microorganisms. The contribution of each conversion highly depends on the chemistry of a pesticide, field conditions including meteological factors, plants, and population of microorganisms. Diethofencarb (Powmyl, isopropyl 3,4-diethoxycarbanilate) is a carbamate fungicide possessing preventive and curative activity against benzimidazole resistant strains of *Botrysis* spp. on grapes and vegetables (*1*). Because *N*-phenyl carbamate is known to be resistant to hydrolysis under the natural conditions (*2*) and sunlight irradiance is usually shaded by plants, the examination of soil metabolism was considered to be of importance in assessing an environmental fate of diethofencarb. Sakata et al. (*3*) showed its rather rapid degradation under the aerobic conditions with half-lives of 0.3−6.2 days in Japanese soils and reported the very unique nitration reaction at the 6-position of the 3,4-diethoxyphenyl moiety. The similar nitration of pesticide has been recently reported for the phenyl ring of famoxadone and pyrimethanil (*4*, *5*). Although many reactions where microorganisms and abiotic soil components participate are known in the soil environment, oxidation and reduction are predominant and nitration is only known as the clay-catalyzed reaction (*6*, *7*). Cornelis et al. have prepared montmorillonite treated with transition metal nitrates such as ferric nitrate (Clayfen) (*8*), which efficiently catalyzes nitration of aromatic molecules under the mild conditions. The reaction mechanism is still obscure, but nitrosonium ion and aromatic cation radical were proposed to be involved.

We have investigated the effect of soil characteristics such as clay species, soil pH, organic content, and moisture content as dominant factors controlling nitration of diethofencarb, and the possible involvement of microbial metabolism was also examined together with requirement of oxygen in nitration. Furthermore, a series of ^{14}C-labeled diethofencarb analogues were synthesized and subjected to nitration in the presence of kaolinite clay and kinetic analysis was conducted to estimate a reaction mechanism.

MATERIALS AND METHODS

Chemicals. The chemical structures of carbamates used in this study are listed in **Table 1** and **Figure 1**. Diethofencarb (**I**) (isopropyl 3,4-diethoxycarbanilate) and its analogues, **II**−**VII**, were synthesized from the corresponding aniline and isopropyl chloroformate in our laboratory according to the reported method (*1*). Compound **VIII** was prepared from **IV** by treating with boron tribromide in dichloromethane. Compound **VII** was reduced by 0.5% Pd−C to the corresponding aniline delivative, which was further reacted with methyl iodide in the presence of potassium carbonate to prepare **IX** or with acetic anhydride in the presence of a catalytic amount of pyridine to prepare **X**. These carbamates were purified by preparative silica gel thin-layer chromatography (TLC) with their chemical purities determined to be >99% by high-performance liquid chromatography (HPLC) and their chemical identities were confirmed by ^{1}H NMR and liquid chromatography−mass spectrometry (LC-MS). [^{14}C]**I** uniformly labeled in the phenyl ring (8.9 MBq/mg, radiochemical purity > 99%) and [^{14}C]**II**−**X** labeled at the isopropyl methine carbon (8.2−10.8 MBq/mg, radiochemical purity > 99%) were similarly prepared in our laboratory. The nitrated

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* To whom correspondence should be addressed. E-mail: kodaka@sc.sumitomo-chem.co.jp.

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Table 1. Chemical Structures of Carbamates with Spectroscopic and Chromatographic Properties

		^{1}H NMR[a]				R_f[c]	
chemicals	R	R	phenyl (*J*, Hz)	LC-MS (*m/e*)	R_t (min)[b]	A	B
I	3,4-$(OC_2H_5)_2$	4.07 (4H, ddd) 1.29 (6H, d)	7.16 (1H, d, $J = 7.2$) 6.75 (2H, dd, $J = 7.5/8.1$)	268 (M + H)	26.2	0.67	0.56
II	H		7.38 (2H, d, $J = 8.4$) 7.30 (2H, dd, $J = 6.9–8.1$) 7.05 (1H, dddd, $J = 7.3/1.2$)	180 (M + H) 138	24.0	0.73	0.68
III	4-CH_3	2.30 (3H, s)	7.25 (2H, d, $J = 8.1$) 7.10 (2H, d, $J = 8.1$)	194 (M + H) 152	26.1	0.77	0.70
IV	4-OCH_3	3.80 (3H, s)	7.28 (2H, d, $J = 8.3$) 6.84 (2H, d, $J = 8.3$)	210 (M + H) 168	23.2	0.76	0.64
V	4-Cl		7.35 (2H, d, $J = 7.0$) 7.25 (2H, d, $J = 7.0$)	212 (M − H)	28.0	0.66	0.63
VI	4-CN		7.60 (2H, d, $J = 7.8$) 7.51 (2H, d, $J = 7.8$)	203 (M − H)	25.0	0.55	0.53
VII	4-NO_2		8.20 (2H, d, $J = 12$) 7.54 (2H, d, $J = 12$)	223 (M − H)	26.0	0.67	0.66
VIII	4-OH	4.71 (1H, bs)	7.15 (2H, d, $J = 8.1$) 6.73 (2H, dt, $J = 8.7/3.5/2.1$)	194 (M − H)	21.0	0.32	0.34
IX	4-$N(CH_3)_2$	2.91 (6H, d, $J = 3.1$)	7.22 (2H, d, $J = 8.4$) 6.70 (2H, dd, $J = 8.3/2.2$)	223 (M + H)	15.7	0.68	0.03
X	4-$NHCOCH_3$	7.21 (NH, bs) 2.2 (3H, t, $J = 2$)	7.42 (2H, d, $J = 7$) 7.32 (2H, d, $J = 6.9$)	237 (M + H) 195	20.2	0.25	0.20

[a] In chloroform-d_1, δ ppm vs TMS. Chemical shifts of aromatic protons are described. [b] HPLC retention time. [c] In solvent system of A, chloroform/methanol (9:1, v/v), and B, toluene/ethyl acetate/acetic acid (5:7:1, v/v/v).

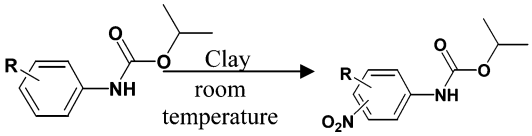


Figure 1. Chemical structure of carbamates and their nitro derivatives in clay-catalyzed nitration.

derivative of **I** at the 6-position of the phenyl ring (**XI**) was synthesized as previously reported (*3*). The similar nitrated derivatives of **II**−**X** formed via reaction on the kaolinite surface were synthesized by treating with NO_2BF_4 in dichloromethane under nitrogen atmosphere in our laboratory.

$K^{15}NO_3$ and $Na^{15}NO_3$ were purchased from ISOTEC Inc. (Miamisburg). 2,2,6,6-Tetramethyl-4-piperidiol (4-OH-TEMP), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), triethylamine, *N*-*tert*-butyl-α-phenylnitron, acetochlor (2-chloro-2′-ethyl-6′-methyl-*N*-ethoxymethylacetanilide), alachlor (2-chloro-2′,6′-diethyl-*N*-methoxymethylacetanilide), and the other reagents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Famoxadone (3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione) was a courteous gift from DuPont de Nemource and Company. These chemicals were used without further purification. Kaoline, montmorillonite KSF, and montmorillonite K10 were purchased from Wako Pure Chemical Industries Ltd. Activated earth (montmorillonite) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

Spectroscopy. Fourier transfer (FT) NMR spectra were measured in dilute solutions of chloroform-d_1 using tetramethylsilane (TMS) as an internal standard ($\delta = 0.00$ ppm) with a Varian unity 300 NMR spectrometer at 300 MHz. API in a positive ion mode and single ion mass (SIM) spectra were obtained with a Hitachi M-1000 mass spectrometer. FTIR spectra were recorded with a FTIR Perkin-Elmer Spectrum One over the range of 4000−650 cm^{-1} using a universal ATR sampling accessory. Samples were mounted on the accessory and scanned with an optical resolution of 2 cm^{-1}.

Radioassay. The radioactivity in extracts of soils and clays was individually determined in duplicate by mixing each aliquot with 10 mL of Packard Emulsifier-Scintillator Plus in a low potassium glass vial followed by liquid scintillating counting (LSC) with a Packard model 2000CA liquid scintillation analyzer. The radioactivity bound to soil or clay was quantified by combusting each aliquot (100 mg) with a Packard 307 sample oxidizer prior to LSC measurement. The recovery of ^{14}C in a sample oxidizer was >90% when 9 mL of Packard Carb-CO_2 absorber and 15 mL of Packard Permaflour oxidizer scintillator were used. The background levels were 0.8 Bq, and more than 1.7 Bq of radioactivity was quantitatively determined.

Chromatographies. The extracts from each sample were individually analyzed by reversed phase HPLC. A Hitachi L-6200 liquid chromatograph equipped with a Sumipax ODS A-212 column (5 μm, 6 mm i.d. × 15 cm, Sumika Analytical Service, Ltd., Osaka) was operated at a flow rate of 1 mL min^{-1}. The composition of the mobile phase was changed stepwise as follows: 0 min, %A (acetonitrile)−%B (0.01% trifluoroacetic acid), 10:90; 0−30 min, linear, 90:10 at 30 min; 30−45 min, isocratic. The UV absorbance at 254 nm was monitored with a Hitachi model L-4000 UV detector. The radioactivity of column effluent was monitored with a Packard Flow-one/Beta A-100 radio detector equipped with a 500 μL liquid cell using Ultima-Flo AP (Packard) as a scintillator. Each ^{14}C peak was identified in HPLC cochromatography by comparing its retention time with those of nonradiolabeled authentic standards detected by the UV detector. The typical retention times of **I** and related compounds are listed in **Table 1**.

Precoated silica gel 60F_{254} chromato plates (20 cm × 20 cm, 0.25 mm thickness, E. Merk) were used in TLC for analytical and preparative puposes. The R_f values of **I** and related compounds are also listed in **Table 1** together with the solvent systems used in development. The nonradiolabeled reference standards were detected by ultraviolet light at 254 nm. Autoradiograms were prepared by exposing the TLC plate to BAS-III_S Fuji imaging plate (Fuji Photo Film Co., Ltd.) for several hours. The radioactivity was quantified by Bio Imaging Analyzer BAS-1500 (Fuji Photo Film Co., Ltd.).

Formation of XI in Soils and Clays. The 14 Japanese soils collected from the top 10 cm of each ground were passed through a 2 mm sieve prior to use to remove stones and plant debris. Their physical and chemical properties are listed in **Table 2**. Ten grams of each soil on a dry weight basis was taken into a glass beaker (3.5 cm diameter) and preincubated in darkness at 20 ± 1 °C for 14 days by using a M-1200ES incubator (Advantec Toyo Co., Ltd.). After preincubation, each soil sample was dropwise fortified with 50 μL of acetonitrile solution of [^{14}C]**I** at a rate of 1 ppm by using a microsyringe. The moisture content was adjusted to 50% of the maximum water holding capacity by appropriately adding distilled water. The clays were suspended using a minimal volume of water and air-dried without adjustment of moisture

Table 2. Soil and Clay Characteristics with Amounts of XI Formed[a]

		physicochemical properties of the test soils						
		texture (%)						
no.	soil name	sand	silt	clay	main clay mineral[b]	pH (H_2O)	organic content (%)	XI[c]
1	Okayama	60	16	14	K3+, A+, C+, I+	6.7	1.3	11.5
2	Noichi	53	24	22	K3+, C2+, I+	6.5	2.6	6.3
3	Fukui	42	26	32	A2+, AV+, K+	5.5	2.7	4.1
4	Tondabayashi	64	17	19	A2+, K2+, M+, Al–Vt+	5.3	2.2	2.2
5	Fukushima	45	32	23	A2+	6.3	2.8	0.8
6	Sapporo	43	32	25	A3+, I2+	5.3	12.0	0.7
7	Ushiku	39	37	24	A4+	7.4	6.2	ND
8	Takarazuka	86	7	7	A3+	6.5	1.6	ND
9	Miyazaki	98.2	0.3	1.5	A3+	6.2	0.3	ND
10	Kumamoto	45	34	21	A2+, K+, M+, I+	6.6	10.7	ND
11	Hyogo	90	5	5	Al–Vt2+, K2+, A+, I+	5.8	<0.1	ND
12	Kasai	66	17	17	A2+, K2+, Vt+, I+, M+	6.6	2.9	ND
13	Tokushima	55	26	19	C2+, I2+, K2+	6.1	2.4	ND
14	Niigata	49	23	27	M3+, A+	5.0	5.3	ND
15	Kaolinite	0	0	100	K	NA	NA	24.7
16	activated earth (montmolinite)	0	0	100	M	NA	NA	18.4
17	Montmorillonite KSF	0	0	100	M	NA	NA	3.4

[a] NA, not analyzed; ND, not detected. [b] K, kaolinite; A, allophane; C, chlorite; I, illite; M, montmorillonite; Vt, vermiculite; Al–Vt, Al–vermiculite; +, clay mineral % in the clay fraction (3+, 50–80%; 2+, 30–50%; +, 10–30%). [c] Amount of XI formed after incubation at 20 °C for 7 days.

content. Each vessel was placed in the incubator and kept at 20 ± 1 °C in darkness.

To examine the effect of soil characteristics on formation of **XI**, the 7 day incubation was first conducted for all soils and three clays. After 7 days, the soil sample was transferred to a centrifugal bottle and extracted with 20 mL of methanol/0.2 M HCl (5/1, v/v) by mechanical shaking for 10 min. After the samples were shaken, centrifugation was conducted for 10 min at 5000 rpm and 4 °C, using a high-speed refrigerated centrifuge (CX-250, Tomy). The extraction was repeated twice in the same manner as above. The supernatant was combined and concentrated under the reduced pressure to approximately 10 mL and used for HPLC analysis. The observed peaks were identified by HPLC cochromatography with the nonradiolabeled reference standards. Because more formation of **XI** was detected in three clays after a 7 day incubation, kaolinite was conveniently used to examine factors controlling nitration of **I**. First of all, the formation profiles of **XI** were examined up to 32 days. Kaolinite equivalent to 1 g on a dry weight basis was taken into the beaker and suspended with 2 mL of pure water (PURIC-MX II, Organo) to prepare a thin layer. After the equivalent was air-dried, it was treated with [^{14}C]**I** in acetonitrile and incubated similarly to the 7 day study. At appropriate intervals, the clay thin layer was extracted and analyzed by HPLC. After extraction, the radioactivity bound to clay was further quantified by combustion analysis to examine the possible formation of bound ^{14}C during incubation.

Effect of Molecular Oxygen on Formation of XI. The effect of oxygen was investigated by the following two ways. Kaolinite is a fine powder, and the diffusion of molecular oxygen would be reduced when the thicker clay layer was used. Therefore, the appropriate amount of kaolinite was added to the glass beaker, suspended in water, and air-dried finally to realize the thickness of the clay layer to become 1, 2−3, and 10 mm. To each layer, [^{14}C]**I** in acetonitrile was dropwise applied using a microsyringe. Second, the atmosphere in incubation was changed to the anaerobic one. The clay thin layer was prepared from a suspension of 1 g of kaolinite in 2 mL of pure water in the bottom of a glass beaker (3.5 cm i.d.). After the water had dried up, it was uniformly treated with [^{14}C]**I** (53.4kBq, 6 μg) in acetonitrile and incubated under anaerobic conditions of N_2:H_2:CO_2 = 8:1:1 (v/v/v) for 7 days in an anaerobic chamber (Air Tec, Japan).

Effect of Moisture Content on Formation of XI. Kaolinite equivalent to 1 g on a dry weight basis was taken into a glass vessel (3.5 cm diameter). The moisture content was adjusted to 0.7, 10, 50, and 100% by adding an appropriate amount of pure water. The dry clay was prepared by air-drying for 3 days and/or heating at 120 °C for 2 h. Each clay thin layer was with a different moisture content uniformly treated with [^{14}C]**I** (8.9kBq, 1 μg) in acetonitrile, covered with Parafilm, and then incubated at 20 ± 1 °C in darkness for 7 days followed by analysis.

Examination of Nitrogen Source. As a nitrogen source, NO_3^- and NH_4^+ are considered most probable in soil. Therefore, their possible incorporation into **I** was examined by using NO_3^- and NH_4^+ free kaolinite. The <2 μm fractions of kaolinite were obtained by sedimentation, and the corresponding homoionic clay was prepared by successive treatment with 1 M $CaCl_2$ in the usual manner. The samples were centrifuged, washed repeatedly with deionized water, and dried at room temperature. The clay thin layer was then prepared from a suspension of 1 g of kaolinite in 2 mL of pure water containing either $K^{15}NO_3$ or $Na^{15}NO_3$ at 0.2% concentration. After the water had dried up, the clay thin layer was uniformly treated with [^{14}C]**I** (53.4kBq, 6 μg), incubated for 7 days at 20 °C, and [^{14}C]**XI** extracted from clay was subjected to MS analysis to examine the incorporation of ^{15}N into XI.

In addition, the effect of metal cation on clay surface was examined for montmorillonite KSF by using Ba^{2+}, NH_4^+, and Na^+ homoionic clays being prepared with the corresponding 1 M metal chloride solutions. The excess metal chlorides together with Cl^- were removed by repeated washing of the clay with water. The clay thin layer was similarly prepared from these homoionic clays and incubated to examine the degradation of **I**.

Investigation of Reaction Mechanism. Nitration is known to proceed via cationic or radical intermediate with a different regioselectivity. To examine the possible involvement of a radical intermediate, some radical quenchers were utilized in the reaction of **I** in kaolinite. The clay thin layer was prepared from a suspension of 1 g of kaolinite in 2 mL of pure water. After the water had dried up, the clay thin layer was uniformly treated 0.5−1 mL of chloroform containing 0−10 mg of either 4-OH-TEMP, DMPO, triethylamine, or *N*-tert-buthyl-α-phenylnitron, as spin-trap or radical scavengers. Molar ratios of **I** to these chemicals were calculated to be 25−4400. After the solvent had dried up (for 1 h at room temperature), the clay thin layer was uniformly treated with [^{14}C]**I** (53.4 kBq, 6 μg) and similarly incubated, and the amount of **XI** was examined by HPLC analysis of extracts.

Second, the reaction mechanism was investigated by kinetic analysis of nitration by using the structural analogues of **I**. The kaolinite clay film was similarly prepared in the bottom of a glass beaker (3.5 cm i.d.), and after it was air-dried, it was uniformly treated with [^{14}C]**II**−**X** (8.2−10.8 kBq, 1 μg) in acetonitrile followed by incubation at 20 ± 1 °C in darkness. At an appropriate interval, samples were similarly extracted and analyzed by HPLC cochromatography with authentic nitrated derivaties. For these analogues, the rate of nitration was calculated assuming first-order kinetics. In the case of **VII**−**X**, the reactions in kaolinite clay layer were also conducted at 30, 50, and 80 ± 1 °C.

Furthermore, the semiempirical AM1 molecular orbital (MO) calculations (*9*) were applied to **I** to estimate possible position of nitration, using the WINMOPAC program (version 2.0, Fujitsu Ltd.). The initial molecular geometry of **I** was derived from the standard values of bond lengths and angles together with appropriate torsional angles. All of the geometrical parameters were fully optimized by using an EF routine, and the energy levels of the highest occupied MO (HOMO) and the lowest unoccupied MO (LUMO) were estimated together with their distribution at the phenyl ring. The similar calculations were also conducted for the cation radical of **I** and the several nitrogen oxide species possibly to be involved.

Furthermore, to investigate the possible nitration for other pesticides, acetochlor, alachlor, and famoxadone, with the aniline moiety not substituted with an electon-withdrawing group such as Br and Cl, were subjected to the clay reaction at 5 ppm. After 20−30 days of incubation at 20 °C, they were similarly extracted and analyzed by HPLC and the incorporation of nitro group was examined by SIM mass spectra.

To investigate the mode of interaction of **I** with the clay surface, IR spectra of **I** adsorbed on kaolinite and montmorilonite were measured as follows. After clays were sieved at 50 μm, a 50 mg aliquot was individually mixed with 2 mL of 2% chloroform solution of **I** in 3 mL screw-capped glass vials. After 3 days, the slurries were centrifuged at 3000*g* for 10 min, washed with pure water several times, and air-dried. Blank samples treated only with pure solvent were also prepared. Difference spectra were obtained for clay treated with **I** by subtracting the spectra of the corresponding blank clay.

RESULTS

Formation of XI on Soils and Clays. The amounts of **XI** after a 7 day incubation under the aerobic conditions with the moisture controlled are summarized in **Table 2** for the 14 soils tested. In the six soils (nos. 1−6), **XI** amounted to 0.7−11.5% of the applied ^{14}C, while no formation of **XI** was detected in other soils. Among the soil characteristics possibly controlling the nitration of **I**−**XI**, the soil pH seemed unlikely to be of importance. The five soils (nos. 2, 5, 8, 10, and 12) showed similar pH values of 6.3−6.6; only Noichi soil (no. 2) gave the significant formation of **XI** (6.3%), and the insignificant nitration proceeded in the other soils (≤0.8%). The percentage of **XI** formation after 7 days was plotted against organic matter content (%) and percentage of clay content as shown in **Figure 2**. The lesser the organic matter content and the greater the clay content, the more formation of **XI** was clearly observed. These results implied that nitration of **I** would most likely proceed on the clay surface, which was clearly demonstrated by the incubation of **I** in three types of clays. Kaolinite and montmorilonites accelerated formation of **XI**, amounting to 3.4−24.7% of the applied ^{14}C.

To examine the formation profiles of **XI** from **I**, the reaction in the kaolinite treated with [^{14}C]**I** was periodically monitored for up to 32 days, as shown in **Figure 3**. Compound **I** was degraded with a half-life of 5.9 days (r^2 = 0.823, first order) to **XI** with the insignificant formation of other degradates (<6.7%) and bound ^{14}C (<2.4%). The good material balance (96.0−98.1%) showed the insignificant formation of volatile ^{14}C. Because kaolinite used in this study was most likely prepared by rigorous degradation of organic matters and repeated washing with pure water, the nitration was most likely to proceed abiotically on the clay surface.

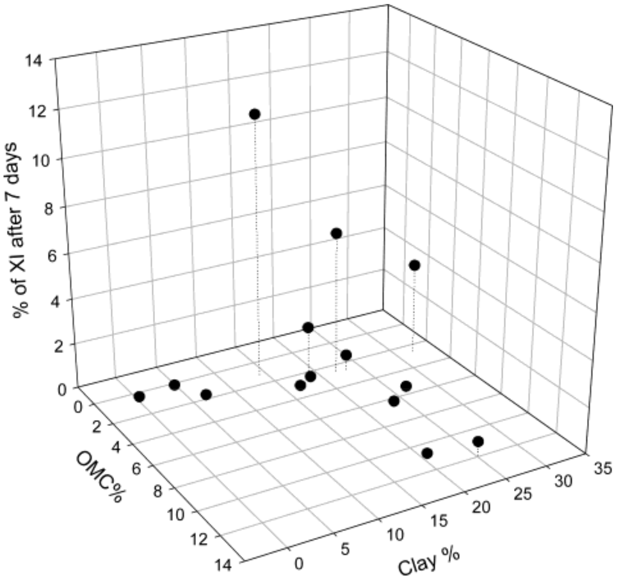


Figure 2. Three-dimensional plot of percent XI vs organic matter content (OMC %) and clay content (clay %).

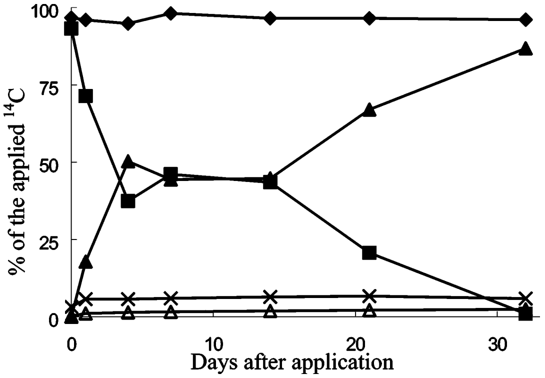


Figure 3. Nitration rate of I on kaolinite. Total ^{14}C (◆), I (□), XI (▲), others (×), and bound ^{14}C (△).

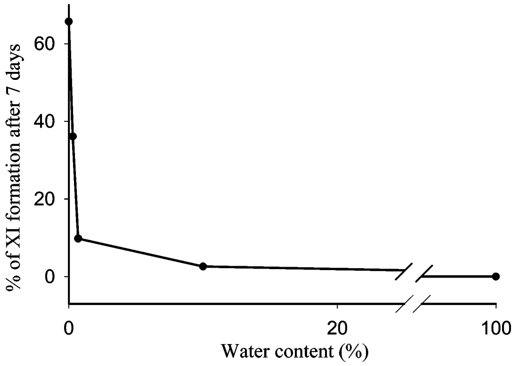


Figure 4. Effect of water content on formation of XI in the kaolinite thin layer.

To get more information on the reaction profiles, the effect of moisture content was examined on kaolinite at 1 ppm of **I**, as shown in **Figure 4**. Dryness by heating in the oven at 120 °C for 2 h was conveniently considered to give the 0% water content. After air dryness, the water content of kaolinite was gravimetrically determined to be 0.3%. After 6 days, **XI** amounted to 65.7% in the oven-dried kaolinite, but its formation was retarded with an increase of a water content. Because the water adsorbed onto the clay surface cannot be completely removed under the tested conditions (*10*), the minimum coverage of water molecules was important for the clay-catalyzed nitration of **I**.

Because the previous soil metabolism study of [^{14}C]**I** using Japanese soils showed no formation of **XI** under the anaerobic conditions by purging with $N_2/CO_2/H_2$ (8:1:1, v/v/v), the effect

Table 3. Controlling Factions of Formation of XI in Clay

atmosphere	thickness of layer (mm)	additives	% of the applied $^{14}C^a$ I	XI
		kaolinite		
aerobic	10	*b*	80.3	13.4
aerobic	2–3	*b*	55.4	37.3
aerobic	1	*b*	30.5	60.9
anaerobic^c	1	*b*	96.0	ND
aerobic	1	KNO_3	37.9	54.5
aerobic	1	$K^{15}NO_3$	28.4	65.0
aerobic	1	$Na^{15}NO_3$	0.9	90.5
		montmorillonite KSF		
aerobic	1	*b*	80.3	9.3
aerobic	1	$CaCl_2$	62.3	25.5
aerobic	1	$BaCl_2$	39.1	42.6
aerobic	1	NH_4Cl	23.7	32.7
aerobic	1	NaCl	39.7	49.4

^{a} Incubation at 20 °C after 7 days. ^{b} Not treated. ^{c} In the atmosphere of $N_2/CO_2/H_2$, 8:1:1 (v/v/v).

of molecular oxygen was first examined for the kaolinite treated with [^{14}C]**I** at 1 ppm. By changing the thickness of the kaolinite layer from 10 to 1 mm, the amount of **XI** formed after 7 days increased from 13.4 to 60.9% as summarized in **Table 3**. Assuming the homogeneous distribution of **I** in the kaolinite layer, the thicker layer would reduce the diffusion of molecular oxygen, which may result in less reaction with O_2. When the kaolinite treated with [^{14}C]**I** was incubated under the anaerobic atmosphere ($N_2/CO_2/H_2$, 8:1:1), no formation of **XI** was observed. These results showed the importance of molecular oxygen for the nitration of **I** on the clay surface.

Finally, the source of nitrogen was examined. The nitrate ion was added to the kaolinite layer prior to incubation, since it is one of the most abundant inorganic ions in the soil environment. The excess amount of potassium nitrate scarcely affected the reaction as shown in **Table 3**, and moreover, MS analysis around $m/z = 312$ could not demonstrate any ^{15}N incorporation into **XI**. In the presence of $Na^{15}NO_3$, the nitration was greatly enhanced but without incorporation of ^{15}N into **XI**, as also evidenced by MS analysis. Although the direct evidence could not be obtained, these results were likely to show that some nitrogen source was firmly attached to the clay surface and participated in nitration.

By the way, the countercation on the clay surface is known to affect the surface environment such as surface acidity; the several homoionic clays were examined as thin layers (at a thickness of 1 mm). In all homoionic clays of montmorillonite KSF, the extent of nitration increased and **XI** amounted to 25.5−49.4% of the applied ^{14}C after 7 days at 20 °C (**Table 3**). There was no clear relationship between the amount of **XI** and cation characterization such as ion radius (Na^+, 1.16 Å; Ba^{2+}, 1.49 Å; Ca^{2+}, 1.14 Å) when six H_2O molecules are coordinated (*11*). These results showed that nitration of **I** in soil abiotically proceeded on the clay surface under the dry condition via reaction with a nitrogen source tightly bound to the clay surface in the aid of molecular oxygen.

Nitration of Carbamate Analogues on Kaolinite. Nitration of **I** and the related analogues (**II**−**X**) in the air-dried kaolinite thin layer (1 mm thickness) at 20 °C under the aerobic conditions were determined. After 7 days, the HPLC analysis of clay extracts showed the appearance of the new peaks as with incubation. The corresponding nitrated derivatives were identified by HPLC cochromatography with authentic standards. The authentic standards of nitrated derivatives were synthesized in our laboratory, and their structure was confirmed by LC-MS and ^{1}H NMR spectra, as summarized in **Table 4**. Isopropyl carbanilate (**II**) labeled with ^{14}C at the isopropyl moiety was degraded with a half-life of 81 days, leading to formation of nitrated derivatives with insignificant bound residues (0.6% after 30 days). The amounts of 2- and 4-NO_2 derivatives were 6.0 and 4.2% of the applied ^{14}C, respectively, with almost the constant *o*/*p* (2-/4-) ratio of 1.5∼2:1 at any sampling time. The substitution of electron-donating groups such as methyl (**III**) and methoxy (**IV**) at 4-position of the phenyl ring enhanced the nitration, and the 2-NO_2 derivatives were only produced. In contrast, introduction of electron-withdrawing groups such as Cl (**V**), CN (**VI**), and NO_2 (**VII**) at the 4-position inhibited nitration even when the reaction was conducted at higher temperatures (50 and 80 °C). When the OH (**VIII**), $N(CH_3)_2$ (**IX**), or $NHCOCH_3$ (**X**) group was introduced at the 4-position, these carbamates underwent nitration at the 3-position of the phenyl ring. Similarly, the formation of the nitrated derivatives of acetochlor and famoxadone was confirmed through the incubation in kaolinite. After incubation at 20 °C for 20−30 days, SIM-MS of the kaolinite extracts exhibited ion peaks corresponding to each pesticide (acetochlor, *m*/*e* 269; famoxadone, *m*/*e* 374) together with those possessing the mass greater than the parent by *m*/*e* of 45 (= NO_2). In the case of famoxadone, the two peaks observed in SIM-MS were considered to correspond to the *o*- and *p*-NO_2 derivatives. Any new MS peak corresponding to the nitrated derivative of alachlor could not be detected through incubation even for up to 30 days.

To investigate the nitration process more in detail, the formation rate (*k*) of the nitrated derivative of each analogue was calculated assuming the pseudo-first-order kinetics based on HPLC analysis of extracts, as listed in **Table 4**. Because the change of reaction rate with a regioselectivity of nitration seemed to imply involvement of an electrophilic substitution mechanism, the relative reaction rates to that of (**II**) (k/k_0) were plotted against the Hammett σ^+ values, as shown in **Figure 5**. The linear regression analysis gave the following relationship.

$$\log(k/k_0) = -7.746\,\sigma^+ + 0.7211\ (r^2 = 0.86,\ n = 5)$$

The large negative slope ($\rho = -7.746$) strongly suggested the electrophilic substitution mechanism being known for the usual nitration by nitronium ion.

By the way, one electron oxidation of an organic molecule has been known for various kinds of clays (*12*). Therefore, the possible involvement of radical mechanism was examined. The radical scavengers or spin-trap reagents were added to kaolinite at three levels of concentration prior to incubation with [^{14}C]**I**. Either triethylamine as a radical scavenger or 4-OH-TEMP as a quencher of singlet oxygen was only effective at a molar ratio to **I** of 2800−4400 (addition of 10 mg), while DMPO and *N*-*tert*-butyl-α-phenylnitron as quenchers of hydroxyl and/or superoxide anion radicals retarded the nitration of **I** at a much lower molar ratio of 25−39, as shown in **Table 5**. Although the retardation of nitration in the presence of the extremely more amounts of these reagents might simply be caused by their coverage of the clay surface inhibiting the adsorption of **I** to active sites, more effectiveness of DMPO and *N*-*tert*-butyl-α-phenylnitron was likely to show the involvement of hydroxyl and/or superoxide anion radicals in nitration.

Because these carbamates underwent nitration through binding to clay surfaces, the mode of interaction of **I** with clay was studied by IR with an ATR mode. **Figure 6** shows IR difference spectra of **I**, **I** in kaolinite, and montmorillonite KSF at 1750−1100 cm^{-1}. The absorption due to stretching of the carbonyl

Table 4. Kinetic Analysis of Nitration of I and Its Analogues and Structure of Formation Products

R	nitrated derivative	R_t (min)[b]	TLC R_f[c] A	TLC R_f[c] B	^{1}H NMR[d] of aromatic moiety: substituent	^{1}H NMR[d] of aromatic moiety: ring-H	MS (*m*/*e*)	rate of formation (μmol/s)	σ^{+}[f]	k/k_0[g]
I	XI	33.1	0.80	0.71	4.20 (4H, ddd) 1.35 (6H, dd)	8.21 (1H, s) 7.68 (1H, s)	313 (M + H) 227	2.30×10^{-7}		
II	2-NO$_2$	28.8	0.80	0.72		8.58 (1H, d, *J* = 8.4/0.9) 8.20 (1H, dd, *J* = 9.0/1.2) 7.62 (1H, ddd, *J* = 8.7/1.5) 7.10 (1H, ddd, *J* = 7.5/1.5)	225 (M + H) 183, 139	1.49×10^{-10}	0	1
	4-NO$_2$	26.1	0.67	0.66		8.20 (2H, d, *J* = 12) 7.54 (2H, d, *J* = 12)	225 (M + H) 177	1.10×10^{-10}		
III	2-NO$_2$	30.7	0.80	0.74	2.37 (3H, s)	8.44 (1H, d, *J* = 8.7) 7.99 (1H, d, *J* = 1.5) 7.44 (1H, dd, *J* = 8.7/1.8)	239 (M + H) 197, 153	0.90×10^{-7}	–0.17	398
IV	2-NO$_2$	28.6	0.82	0.69	3.85 (3H, s)	8.46 (1H, d, *J* = 9.3) 7.65 (1H, d, *J* = 3) 7.23 (1H, m, *J* = 12/3)	255 (M + H) 213, 169	1.33×10^{-7}	–0.27	598
V	*a*	NA[e]	NA[e]	NA[e]		NA[e]	NA[e]	NA[e]	NA[e]	NA[e]
VI	*a*	NA[e]	NA[e]	NA[e]		NA[e]	NA[e]	NA[e]	NA[e]	NA[e]
VII	*a*	NA[e]	NA[e]	NA[e]		NA[e]	NA[e]	NA[e]	NA[e]	NA[e]
VIII	3-NO$_2$	27.9	0.61	0.55	6.51 (1H, bs)	8.18 (1H, d, *J* = 3) 7.61 (1H, dd, *J* = 7/3) 7.12 (1H, d, *J* = 9)	239 (M – H)	5.22×10^{-7}	–0.37	2310
IX	3-NO$_2$	29.8	0.68	0.80	2.8 (6H, s)	7.81 (1H, d, *J* = 3) 7.47 (1H, d, *J* = 9.3) 7.02 (1H, d, *J* = 8.7)	268 (M + H)	1.05×10^{-7}	–0.83	463
X	3-NO$_2$	26.5	0.40	0.26	7.63 (NH, bs) 2.20 (3H, s)	8.49 (1H, d, *J* = 9.3) 8.43 (1H, d, *J* = 2.4) 7.72 (1H, d, *J* = 9.3/2.4)	282 (M + H)	0.317×10^{-8}	0	14

[a] No reaction was observed at 20, 50, and 80 °C. [b] HPLC retention time. [c] In solvent system of A, chloroform/methanol (9:1, v/v), and B, toluene/ethyl acetate/acetic acid (5:7:1, v/v/v). [d] In chloroform-d_1, δ ppm vs TMS. Chemical shifts of aromatic protons are described. [e] Not analyzed. [f] Hammett σ plus. [g] k, formation rate of the nitrated derivative; k_0, formation rate of the nitrated derivative of II.

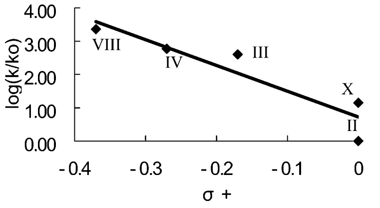


Figure 5. Hammett plot for the nitration of the carbamate analogues.

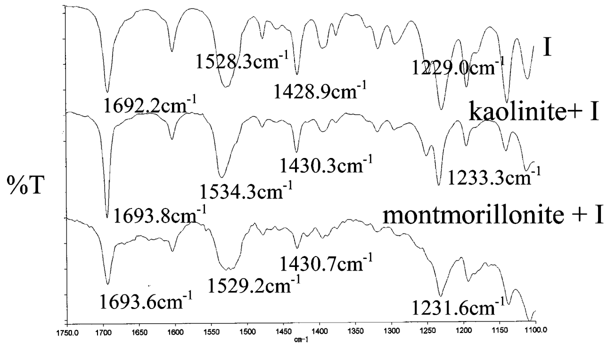


Figure 6. IR spectra of I in clays.

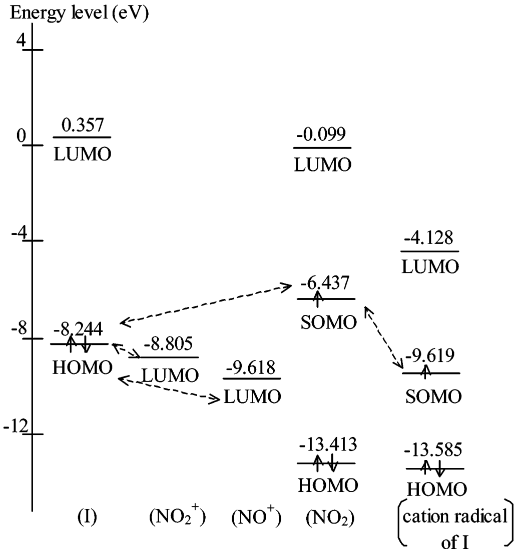


Figure 7. Energy levels of HOMO, SOMO, and LUMO of I and related species estimated by the AM1 MO calculation.

group (amine **I** band) was constantly observed at 1693 cm^{-1}, and the N−H bending at 1602 cm^{-1} did not shift irrespective of adsorption to clays. The broad absorption around 1530 cm^{-1} consisting of C−N and C=C stretching was observed in every case but with its shift to a higher frequency by 6 and 1 cm^{-1} for kaolinite and montmorillonite, respectively. The plane deformation mode of the COO and NHCO moieties was found at ca. 1230 cm^{-1}, and the corresponding adsorption in kaolinite clealy shifted to a higher frequency by 4 cm^{-1}. These results together with the chemical structure of **I** may imply that **I** is adsorbed onto the clay surface by interaction through the carbamate moiety, possibly via ion−dipole interaction between C=O group and exchangeable metal cation on clay (*13*); hence, the phenyl ring would be located parallel to the clay surface.

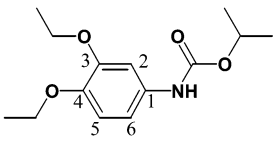
AM1 Calculation. On the basis of the fully optimized structure, the energy levels of HOMO and LUMO (also singly occupied MO, SOMO, for the cation radical of **I**) were estimated as shown in **Figure 7**. In general, MOs with a smaller energy gap and more overlap of orbitals are considered to more effectively interact with each other leading to formation of a new bond. From this viewpoint, the reaction of **I** or its cation

Table 5. Yields of XI on Kaolinite Treated with Radical Quenchers

	XI, % of the applied ^{14}C after 7 days			
additives (mg)	triethylamine	4-OH-TEMP	*N*-*tert*-butyl-α-phenylnitron	DMPO
0	56.3	54.0	52.5	73.8
0.1	41.3 (44)^a	70.9 (28)^a	23.4 (25)^a	11.4 (39)^a
1	31.4 (441)^a	54.6 (283)^a	9.2 (251)^a	7.7 (394)^a
10	ND^b (4406)^a	0.7 (2834)^a	ND^b (2514)^a	0.2 (3938)^a

^{a} Molecular ratio of additives to I. ^{b} ND, not detected.

Table 6. Reaction Indices of I Estimated by the AM1 MO Calculation



	C1	C2	C3	C4	C5	C6
f_N	0.608	0.217	0.059	0.529	0.146	0.118
f_R	0.453	0.119	0.137	0.475	0.111	0.219
f_E	0.298	0.020	0.215	0.420	0.075	0.320

radical with NO^+ or NO_2^+ would be most probable. Because it was very difficult to computationally estimate these reactions along with reaction coordinates, the usual reaction indices f_N (nucleophilic), f_R (radical), and f_E (electrophilic) for **I** were conveniently calculated by using the distribution of HOMO and LUMO, as listed in **Table 6**. These results mean that the nitration is most likely to proceed at the 6-position of the 3,4-diethoxyphenyl ring according to the electrophilic reaction mechanism, usually known for NO_2^+ or NO^+. This estimation was in agreement with the real reaction in soils and clays. However, the possibility of a reaction with some radical species resulting in nitration also at the 6-position could not be excluded. In the case of the radical cation of **I**, AM1 calculation gave the spin distribution as follows: 17 (C1), 22 (C2), 9.9 (C3), 13.8 (C4), 3.6 (C5), and 5.9% (C6). If the cation radical of **I** is involved in nitration as propared for nitration with clay-supported metal nitrates (*7*), it would less favorably proceed at the 6-position, which disagrees with the experimental results. Therefore, AM1 calculations only suggested that nitration of **I** would proceed via electrophilic mechanism of NO_2^+ or NO^+ or via reaction with some radical species on clay surfaces.

DISCUSSION

Both the qualitative analysis on the relationship between nitration of **I** with soil characteristics and the enhanced formation of **XI** in pure kaolinite and montmorillonite clays strongly suggested the abiotic clay-catalyzed process. The catalytic behavior of clays is well-known and has been utilized for many types of organic syntheses (*7*, *14*). In relation to soil metabolism of pesticides, the involvement of catalytic reactions on clay surface has been reported for hydrolysis of organophosphorus pesticides (*15*, *16*) and carbamates (*17*, *18*) and hydration of cyano group of fenpropathrin (*19*), where the Brönsted acidity on the clay surface originating from the proton dissociation of bound water around the exchangeable metal cations had a great role in the reactions. The lesser water content of clays is known to make a water molecule coordinated to a counter metal cation more reactive for nucleophilic attack (*20*) and to extremely increase the surface acidity nearly up to 90% H_2SO_4 (*12*) under the dry conditions. When the thin layer of kaolinite was prepared by air drying, the water content was determined to be about 0.3%; hence, the similar acid-catalyzed reaction would be most likely to proceed for **I** on the surface. By the way, the strong acid such as sulfuric acid is usually used for nitration of an aromatic ring in organic synthesis (*21*), which might be in agreement with the acidic condition of the clay surface. Nitration proceeds via reaction with either nitrosonium or nitronium ion and the *o*/*p* isomer ratio is known to be characteristic to these reactive cations. In the former case, the *p*-NO_2 derivative is dominant, while nitronium ion affords the compounds with an *o*/*p* ratio of 2:1. In the case of kaolinite-catalyzed niration of **II**, this ratio was 1.5−2:1, indicating that the usual mechanism via nitronium ion would operate in our system. For **III** and **IV**, whose 4-positions were blocked by electron-donating substituents, only 2-NO_2 derivatives were formed. In the case of **VIII**−**X** whose substituents at the 4-position have lone pair electrons, the *o*-position to these substituents (3-position) was selectively nitrated. Furthermore, the Hammett equation estimated for these carbamates gave the large negative ρ value, indicating that the nitration on the kaolinite surface was an electrophilic reaction. Thus, the obtained regioselectivity and Hammett analysis imply that nitration may proceed similarly as the usual nitration including NO_2^+ ion rather than NO^+ as an active species. Incidentally, Cornélis et al. (*8*) have reported the efficient catalyst "Clayfen" for nitration of organics, which was an acidic K10 clay treated with ferric nitrate. "Clayfen" affords the *o*/*p* isomer ratio of 0.81:1 for phenol different from the usual nitration, and they proposed a cation radical mechanism. A phenol was considered to be oxidized by clay to form a cation radical, which reacted with NO_2 being produced from reaction of NO^+ with NO_3^- and/or cleavage of a covalent nitrate complex. By thermal analysis of the several metal nitrates on montmorillonite K10, nitrogen oxides were reported to be produced at lower temperatures than that in the absence of clays (*22*).

In our study, nitration of **I** proceeded only at the 6-position of the 3,4-diethoxyphenyl ring. The AM1 calculations of **I**, its cation radical, NO_2^+, and $NO_2^\bullet$ showed that the reaction of **I** with NO_2^+ was energetically favorable but that either with $NO_2^\bullet$ or with the reaction of cation radical of **I** with $NO_2^\bullet$ could not be excluded. However, the spin distribution at the phenyl ring of the cation radical suggested the unfavorable reaction at the 6-position, and the f_E and f_R indices for **I** demonstrated that the 6-position was most probable in nitration. Furthermore, the pretreatment of kaolinite with triethylamine inhibited the reaction. This basic reagent as a radical quencher can neutralize acidic sites on the clay surface; hence, this retardation could not be simply used to clarify the reaction mechanism. The inhibition of nitration on kaolinite by DMPO and *N*-*tert*-butyl-α-phenylnitron showed the possible involvement of radical process by hydroxyl and/or superoxide anion radical and that the radical sites were only deactivated by coverage of excess reagents, indicating that the radical species were not free but bound to the clay surface. The presence of molecular oxygen was found prerequisite for nitration as previously shown in soil metabolism study of **I** (*3*). Larson and Hufnal (*23*) have proposed the possible formation of hydroperoxy radical ($HO_2^\bullet$) on the clay surface via electron transfer from an incorporated transition metal to molecular oxygen. In general, clays are known to have either some defects in lattice where aluminum is substituted with a transition metal such as iron or metal oxides and hydroxides attacked thereon. Although the origin of nitrogen could not be identified in our study, the nitrogen source was most likely to be tightly bound to the clay surface since the clays used in this study showed the nitration activity even after rigorous washing of water or exchanging cations using metal chlorides. Therefore, if $NO_2^{\bullet}$ is formed on the clay surface, the abundant inorganic nitrate ion (*24*, *25*) might be most probably coordinated to such sites (radical mechanism). This process would favorably proceed under the dry condition minimizing side reactions with adsorbed water molecules, but at the same time, the highly acidic environment would also be realized. Therefore, nitronium ion may possibly be formed on the clay surface from nitrate ion (cationic mechanism). The water content dependency and *o*/*p*-regioselectivity with the Hammett analysis supported the cationic mechanism, while the inhibition by spin traps and requirement of O_2 did the radical mechanism. This contradictory result would most likely originate from the heterogeneous reaction environment on the clay surface where the reactive nitrogen species and **I** or the other carbamate were adsorbed.

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