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A novel estrogenic compound transformed from fenthion under UV-A irradiation

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ABSTRACT

The photo-transformed products of fenthion well-known as one of the most photosensitive organophosphorus insecticides and their estrogenic activities were investigated using a yeast two-hybrid assay incorporating the human estrogen receptor α (hERα). We identified fenthion sulfoxide and 3-methyl-4-methylsulfinylphenol (MMS) as the major transformed products and 3-methyl-4-(methylthio)phenol (MMP) as the minor product under UV-A irradiation. Further, significant estrogenic activity was observed in the solution irradiated for 160 min; this activity was evaluated as 18 pM converted to 17β-estradiol (E_2) equivalent concentration. By using authentic standards, it was found that MMP possessed weak estrogenic activity; its activity was evaluated as 1.7×10^{-6} times compared with that of E_2. However, it was also revealed that the activity due to MMP was only 13%. From high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopies, we newly identified a significant estrogenic compound transformed from fenthion, *O*,*O*-dimethyl S-[3-methyl-4-(methylthio)phenyl]phosphorothioate, S-aryl fenthion.

1. Introduction

Fenthion, *O*,*O*-dimethyl *O*-[3-methyl-4-(methylthio)-phenyl] phosphorothioate, is one of the most commonly used organophosphorus insecticides (OPIs). Fenthion has been widely utilized for pest control in agriculture, for example, in the cultivation of rice, fruits, vegetables, beets, etc. [1]. Many researches have been conducted on not only the degradation and/or the transformation processes of fenthion but also on the relative compounds induced by processes occurring under various environmental and biological conditions, for example, hydrolysis [2,3], photolysis [4–6], and biological metabolism [7–9]. Photolysis is a particularly significant process for understanding fenthion degradation in the environment, since fenthion is well-known as one of the most photosensitive insecticide [10]. Further, since fenthion contains two sulfur atoms and one phosphorothio ester bond in its molecular structure, as illustrated in Fig. 1, it can be speculated that the kinetic processes of photolysis are closely related to the wavelength of the irradiation light; moreover, the relative compounds caused by the degradation and/or transformation of fenthion are derived from the oxidation of the sulfur atoms and cleavage reactions of the ester bond. Sakkas et al. [11] investigated fenthion degradation behavior under UV-B irradiation and identified some oxidized and phenolic compounds as the major degradation products. In addition, Sakellarides et al. [6] also investigated fenthion behavior under irradiation with natural sunlight and detected five oxidized and two phenolic compounds using gas chromatograph/mass spectrometer (GC/MS) measurements. They reported identical degraded and/or transformed products such as fenthion sulfoxide and 3-methyl-4-(methylthio)-phenol (MMP); however, their degradation rates were clearly different from those of Sakkas et al. (9.9×10^{-3} min^{-1} [11] and 0.33×10^{-3} min^{-1} [6]). In 2001, Hirahara et al. [12] observed the degradation rate of fenthion under UV-A irradiation, but they did not elucidate the rate constants in terms of first-order degradation reaction since they primarily discussed its behavior on the basis of the half-life values of fenthion concentration. Furthermore, Torrisi and Sortino [13] suggested a new photo-transformation process for fenthion from the viewpoint of photo-isomerization. Therefore, in order to understand the degradation of fenthion due to photolysis and the formation of its relative compounds under irradiation, further investigations are required.

It has been pointed out that certain synthetic chemicals can disrupt the sensitive endocrine systems of humans and other vertebrates by mimicking or inhibiting endogenous hormones such as estrogens and/or androgens [14]. These chemicals are called endocrine disrupting chemicals (EDCs). It has been suspected that pesticides including OPIs could be EDCs, and several researches

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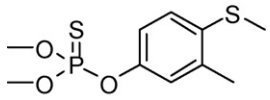


Fig. 1. Molecular structure of fenthion.

have been conducted to verify this possibility. Several reports have been published describing the detection of estrogenic activity in the case of various OPIs *in vitro* bioassays [15–17]. In 2004, Kojima et al. [17] investigated the estrogenic activity of 56 types of OPIs; they found that some of them exhibited estrogenic activity, but they could not detect such activity from fenthion. They also pointed out that the activity of OPIs disappeared when they oxidized to oxon compounds. In 2000, Nishihara et al. [18] reported that most of the estrogenic positive compounds possess a phenol ring with a moiety of appropriate hydrophobicity at the *para* position. As described above, such potential estrogenic compounds could be produced from fenthion after irradiation with UV-B or natural sunlight, and the *para* position of a hydroxyl group, such as the methyl(thio)-group, has a hydrophobic moiety. Hence, we speculate that photo-irradiation may be the cause of the estrogenic activity of a fenthion solution containing some phenolic compounds.

In the present study, we aimed to elucidate the kinetic behavior of fenthion under photo-irradiation on the basis of the first-reaction process, and the UV-A light was tentatively selected as irradiation one, because it has been reported that the intensity ratio of UV-A and UV-B in natural sunlight is ca. 95% and ca. 5%, and the intensity of UV-C can be regarded as almost little on the ground in terms of the absorption by the ozone layer [19,20]. Further, we identified some degraded and/or transformed products induced by the irradiation by using various analyses methods and quantum chemical calculations. In particular, from the viewpoint of the evaluation of estrogenic activity, we attempted to identify the various active compounds induced by irradiation. In addition, we assessed the estrogenic activity of the irradiated solutions and individual compounds detected in the solution using a yeast two-hybrid assay incorporating the human estrogen receptor α (hERα).

2. Experimental

2.1. Chemicals

Fenthion (98% purity), fenthion sulfoxide (99% purity), and MMP (95% purity) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan), respectively. All the solvents were of analytical grade or high-performance liquid chromatographic grade. 3-Methyl-4-methylsulfinylphenol (MMS) was synthesized from the oxidation of MMP in a similar manner as Hirahara's method [5]. A methanol solution comprising equimolar amounts of hydrogen peroxide (0.2 g, 6.49 mmol) was added to a methanol solution of technical-grade MMP (1 g, 6.49 mmol) with magnetic stirring at room temperature for 15 min. The product was purified with silica gel column chromatography by eluting with an *n*-hexane/acetone mixture (7:3). The residue yielded 0.11 g (yield 9.6%) of charcoal brown syrup. The product was characterized by proton nuclear magnetic resonance (NMR) spectroscopy (ECA-500 spectrometer, JEOL Ltd., Tokyo, Japan) and high-resolution (HR) fast atom bombardment (FAB)/MS (FAB + MS; JMS-700 instrument, JEOL Ltd., Tokyo, Japan). NMR spectra were observed in chloroform-*d* solution, and all the chemical shifts were obtained in parts per million downfield from tetramethylsilane (TMS). ^{1}H NMR: δ 7.76 (d, 1H, J = 8.6 Hz, Ar–*H*), 6.88 (dd, 1H, J = 2.3, 8.6 Hz, Ar–*H*), 6.68 (d, 1H, J = 2.3 Hz, Ar–*H*), 2.70 (s, 3H, S–C*H*$_3$), 2.34 (s, 3H, Ar–C*H*$_3$). HR FAB + MS m/z (M+H)$^+$: 171.0462 (intensity, 100%; calculated for $C_8H_{11}O_2S$; 171.0480). Purity 96% (GC).

2.2. UV-A irradiation experiments

Fenthion solution was prepared in distilled water (100 ml) containing 36 μM of fenthion. A small amount of methanol (1%, v/v) was added to each sample in order to increase fenthion solubility. The UV-A irradiation was performed with a 400 W high-pressure mercury lamp (AHH400S, ARION, Saitama, Japan); a glass filter (U360, Hoya Candeo Optronics Corporation, Saitama, Japan) was used to restrict the transmission of wavelengths except from UV-A range (300–400 nm). The details for the light intensity filtered with U360 have been described [21]. The light intensity on the surface of the solution was evaluated at 365 nm (UV-A), at 312 nm (boundary wavelength between UV-A and UV-B), and at 254 nm (UV-C) by a use of a radiometer (ATV-3, Atto Corporation, Tokyo, Japan), and the intensity was observed as follows; 2.8 mW/cm^2 (365 nm), 1.1 mW/cm^2 (312 nm), and not detected (254 nm). During the irradiation, the solution in a glass beaker was mixed using a stirring bar, and the temperature was maintained at 20 °C. Nine solutions were prepared and irradiated for 2.5, 5, 10, 20, 40, 80, 160, 320, and 0 min (blank).

All the samples were consecutively extracted three times with 20 ml ethyl acetate for 10 min using a reciprocating shaker (SR-2s, TAITEC Co., Ltd., Saitama, Japan); Na_2SO_4 was then added to the extracted solution to remove the water. Subsequently, the solvent was evaporated under reduced pressure, and we adequately dried the residue using a stream of nitrogen; it was then re-dissolved in 1 ml dichloromethane. The sample solution (1 ml) was divided into two parts: one part (0.5 ml) was used for GC/MS measurements while the other was used for bioassay.

2.3. Yeast two-hybrid assay for hERα

The estrogenic activities of the irradiated samples and the degraded and transformed products were examined using a yeast two-hybrid assay system developed by Nishikawa et al. [22] and modified using yeast cells (*Saccharomyces cervisiae* Y190); hERα and coactivator TIF2 are introduced into this system.

The expression plasmids for the ligand-binding domain of hormone receptor and pGAAD424-TIF-2 were introduced into the yeast lines that carried the β-galactosidase reporter gene. The assay was adapted to a chemiluminescent reporter gene (for β-galactosidase) method by employing a 96-well culture plate [23]. To measure estrogenic activity in the assay, aliquots from test chemical solutions were incubated for 4 h at 30 °C, where yeast cells were preincubated overnight at 30 °C in a modified synthetic dextrose medium (not containing tryptophan and leucine). After the incubation period, a mixed solution of 2 mg/ml of zymolyase (Zymolyase 20T, Seikagaku, Tokyo, Japan) and a commercial chemiluminescent reporter gene assay kit (Aurora Gal-XE; ICN Pharmaceuticals, Costa Mesa, CA, USA) in the ratio of 5:3, which induces chemiluminescence and enzymatic digestion, was added followed by the addition of a light-emission accelerator reagent. The intensity of the chemiluminescence produced by the released β-galactosidase was measured using a 96-well plate luminometer (Labsystems Luminoskan, Type 391A, Labsystems Research Centre, Finland). Further details of the assay have been published elsewhere [24].

Estrogenic activity was recorded as EC × 10 (10% effective concentration), that is, the concentration of the test compound exhibiting 10% of the activity of blank controls. In this study, the intensity of the estrogenic activity for individual compounds was evaluated by comparison with the EC × 10 value of the positive control, for which we used 17β-estradiol (E_2; >97% purity). The

intensity of the irradiated solution was calculated on the basis of the concentration ratio of the solution rather than the concentration of the compound itself. Further details of the intensities of the irradiated solution and the fractionated solution by high-performance liquid chromatography (HPLC) are described in the literature [25]. The working range of an assay is 31.3–2000 pM for E_2, 1563–100,000 nM for MMP, and 3125–200,000 nM for fenthion, fenthion sulfoxide, and MMS. In the irradiated solutions that included various transformed products, the intensity of the estrogenic activity was evaluated on the basis of equivalence concentration of E_2 (E_2C). E_2C was calculated as follows: E_2C = (EC × 10 of E_2)/(EC × 10 of the irradiated samples).

These samples were filtered through a 0.50 μm disposable syringe filter unit (ADVANTEC DISMIC PTFE, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). They were then subjected to chromatography, and the fractions were collected. In order to identify estrogenic products, all the fractions were assessed for estrogenic activity using a yeast two-hybrid assay.

2.4. Instruments

The standard and irradiated samples were analyzed by GC (HP 6890 Series; Hewlett-Packard, Palo Alto, CA, USA)/MSD (HP 5972A Series; mass selective detector; Hewlett-Packard) on an HP-5 fused silica capillary column (length: 30 m; inner diameter: 0.32 mm; film thickness: 0.25 μm; J&W Scientific, Folsom, CA, USA). The temperature of the injector was 250 °C, and the column temperature was initially maintained at 60 °C for 1 min after which it was raised to 280 °C at a rate of 10 °C/min with a final hold time of 5 min. Helium was used as the carrier gas at a flow rate of 1.3 ml/min, and the column head pressure was maintained at 1.0 psi. The injection volume was 1 μl in the splitless mode. The electron multiplier voltage for MS was 1988 V, and the interface temperature was maintained at 280 °C. The mass spectra of fenthion and its degraded and/or transformed products were obtained by electron impact ionization at a voltage of 70 eV; they were scanned over a range of m/z 50–550 a.m.u. at a rate of 1.5 scans s^{-1}; the ion source temperature was maintained at 250 °C. The transformed products in the irradiated samples were identified by comparing their GC retention times and mass spectra with the standard values, and they were quantified by correlating the ratio of the peak area of the compound of interest to that of the internal standard (pyrene-d_{10}) to the calibration curve of the standard solution. The standard calibration solution containing standard fenthion, fenthion sulfoxide, MMP, and MMS was prepared in dichloromethane.

HPLC was performed on a Shimadzu LC-20AD HPLC system comprising a degasser, binary pump, manual injector equipped with a 1 ml steel loop, UV detector, fraction collector, and data system (Shimadzu Corporation, Kyoto, Japan). HPLC separation was performed on a reverse-phase column with endcapping (C18; particle size: 5 μM; pore size: 100 Å; column length: 250 mm; i.d.: 20 mm, Inertsil ODS-3, GL Sciences, Inc., Tokyo, Japan) employing the same C18 packing guard column (column length: 50 mm; i.d.: 20 mm, GL Sciences, Inc., Tokyo, Japan). The mobile phase comprised acetonitrile/water (50:50, v/v) in an isocratic mode for 10 min; this was followed by a gradient to 100% acetonitrile for 15 min, and finally by an isocratic elution for 50 min. The solvent flow rate was 8 ml/min. The column was maintained at ambient temperatures (20–25 °C). The UV detection measured the absorbance of the effluent at 270 nm. The injection volume was 1 ml.

2.5. Quantum chemical calculation

For three-dimensional molecular modeling of the tested compounds, GaussView ver. 2.1 was used, and their molecular structures were optimized by using Gaussian R 98w ver. 5.4 [26]. By using a keyword in the Gaussian program package, NMR shielding tensors were predicted using the Hartree–Fock method, and the 6-311G basis set was used. They were computed using the continuous set of gauge transformations (CSGT) and the gauge-independent atomic orbital (GIAO) methods. The calculations were performed using a Dell Dimension 8300 micro-computer.

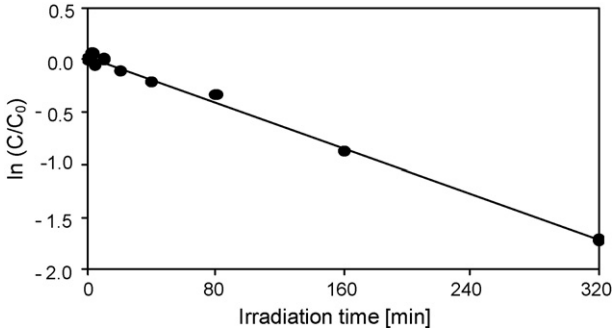


Fig. 2. Change in the concentrations of fenthion during the UV-A irradiation. C_0 is the initial concentration of fenthion, 36 μM. A solid line indicates the following correlation equation: $\ln(C/C_0) = -0.0055 \times$ irradiation time + 0.0282. The square of the correlation coefficient, r^2, is 0.995. Each point represents the mean value of triplicates.

3. Results and discussion

3.1. Degradation rate of fenthion and detected products under UV-A irradiation

In order to elucidate the degradation rate of fenthion on the basis of the first-order reaction, we investigated the concentration change in fenthion in an aqueous solution during UV-A irradiation. Fig. 2 shows the plot of $\ln(C/C_0)$ *vs.* irradiation time. From the value of r^2 and the slope of the correlation equation, it was found that this degradation reaction was of the first order; further, the rate constant k and the half-life $t_{1/2}$ were 0.0055 min^{-1} and 126 min, respectively. As given in Table 1, we compared the obtained k and $t_{1/2}$ with those reported in some literatures [6,11], and it was found that the value of k was considerably larger than that obtained under natural sunlight. However, k is smaller than that obtained under UV-B irradiation. Hence, it appears that the degradation rate depends on the wavelength of the irradiation light, and it is too slow for repeated measurements under irradiation with natural sunlight.

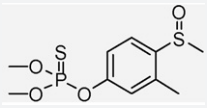
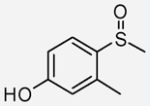
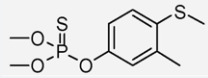
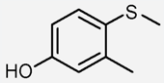
Next, in order to reveal the degraded and/or transformed products induced by fenthion photolysis, the compounds included in the irradiated solution were analyzed, and the concentration change in each compound was observed. Table 2 shows the molecular structures of the compounds detected after irradiation for 320 min and their concentrations. Fig. 3 illustrates the change in the concentration of the identified compounds and the total concentration. The concentrations were corrected by using the recovery ratio [25]. Since the total concentration was maintained as approximately 36 μM, which is the initial concentration of fenthion C_0, the

Table 1
Degradation rate constants, k, and half-lives, $t_{1/2}$.

Authors	Sakkas et al.[a]	Sakellarides et al.[b]	This study
k (min^{-1})	9.9×10^{-3}	0.33×10^{-3}	5.5×10^{-3}
$t_{1/2}$ (min)	70	2016	126
Wavelength (nm)	>290	285–2800	300–400
Light source	Xenon arc	Natural sunlight	High pressure Hg

[a] Reference [11].
[b] Reference [6].

Table 2
Detected compounds and their concentrations in irradiated solution.

Compounds	Molecular structure	Concentration (μM)[a]
Fenthion sulfoxide		24.6
MMS		12.1
Fenthion		5.2
MMP		0.3

[a] These were the corrected concentration used by recovery ratio [25].

mass balance remained constant throughout the photolysis. Therefore, it was believed that the UV-A irradiation primarily induced fenthion sulfoxide and MMS by oxidation at the sulfur atom in fenthion molecule and secondarily induced MMP by cleavage of the phosphorothio ether bond. In the case of MMP, the concentration gradually decreased after irradiation for 80 min. Although many studies on the generation of MMP from fenthion have been reported previously [3,5,6,13], in the present study, MMP was a minor product, which was mostly oxidized to MMS under UV-A irradiation. In addition, it should be pointed out that we also detected various other compounds in the irradiated solution, such as fenthion oxon and some fenthion sulfone compounds; however, we have not discussed these compounds in this paper since their amounts in the solution were insufficient to investigate the kinetic behavior.

3.2. Evaluation of estrogenic activity of the irradiated solution

To estimate the impact of fenthion degradation on ecological and biological systems, especially from the viewpoint of EDCs, we investigated the activity of the fenthion solution irradiated from 0 to 320 min. As shown in Fig. 4, the activity was detected from three samples irradiated at 40 min, 80 min, and 160 min. After we converted the mean intensity of the activity to E_2C, as described in Section 2.3, the activity changes became clearer; for example, it was not initially detected but was then gradually enhanced by UV-A irradiation. However, it appears that further irradiation to the solution induces depression of the activity, or, in other words, reduces it. Then, to elucidate the estrogenic active compounds in

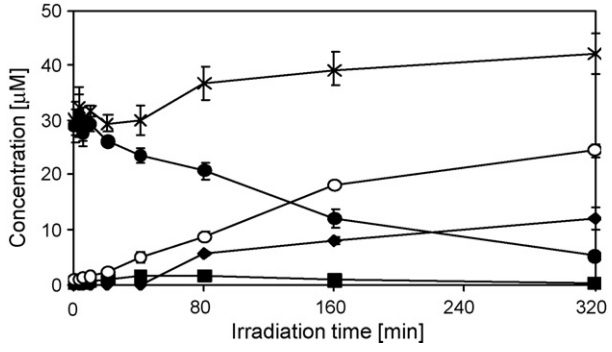


Fig. 3. Change in the concentrations of the compounds during UV-A irradiation. (●) Fenthion, (○) fenthion sulfoxide, (♦) MMS and (■) MMP. Each point represents the mean of triplicates. "×" stands for the summation of the concentrations, i.e., total concentration, corrected by recovery tests [25].

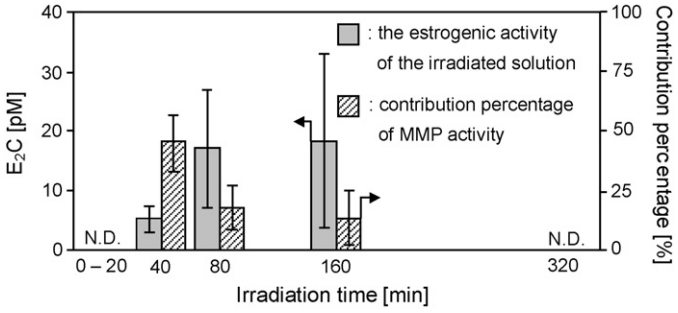


Fig. 4. The time-course of the estrogenic activity of irradiated solutions and the contribution percentage of MMP to the observed estrogenic activity. N.D. stands for "not detected," and each bar represents the mean ± S.D. (n = 3). The estrogenic activity was evaluated as follows: 5 ± 2 pM at 40 min, 17 ± 10 pM at 80 min, and 18 ± 15 pM at 160 min.

the irradiated solution, we quantified the estrogenic intensity of the products that were detected in solutions during the 160-min irradiation by using some synthesized or commercial standards. It was found that MMP, which was a minor degradation product during irradiation, was an active compound and its intensity was evaluated as 1.7×10^{-6} times compared with that of E_2. In contrast, the activity was not detected in the case of fenthion sulfoxide and MMS, which were major degradation products; in addition, the activity was also not detected for fenthion itself by using the present bioassay. With respect to fenthion, its estrogenic activity had been investigated by some researchers, and they also did not detect any significant activity [17,18,27].

Nishihara et al. [18] have pointed out that most of the estrogen active compounds have a phenol ring with a moiety of appropriate hydrophobicity at the *para* position. In the case of MMP, this molecule also has a phenol ring with a 4-(thio)methyl group; this can be regarded as a hydrophobic moiety. Therefore, the structural characteristics of the MMP molecule were considered to be very similar to that indicated by Nishihara et al. With respect to the error bars in Fig. 4, they tended to increase with the irradiation time. A plausible reason for this is that since MMP was a minor product, an experimentally small amount easily induced an increase in the evaluation error.

In order to investigate whether MMP was the only active compound, we calculated the contribution percentage using the follow equation: contribution percentage (%) = {(E_2C of MMP)/(E_2C of original irradiated solution)} × 100.

As shown in Fig. 4, it was estimated that the contribution percentage was 45% (40-min irradiation), 18% (80 min), and 13% (160 min); further, the percentage decreased with increasing irradiation time. It should be noted that the estrogenic activity of the irradiated solution cannot be due to MMP alone; in other words, the solution included the other active compounds induced by UV-A irradiation as well. However, we had already pointed out that MMP was a minor product under this irradiation, and estrogenic activity was not detected with the other compounds detected in the solution. Therefore, it can be concluded that tiny amounts of some unidentified compounds that possess a significant estrogenic activity are present in the solution.

3.3. Identification of a novel estrogenic active compound

In order to elucidate the unidentified compounds, we attempted to fractionate the irradiated solution. Fig. 5 represents an HPLC chromatogram and E_2C of each fractionated sample. Estrogenic activity was detected in the two samples illustrated as F1 and F2 in Fig. 5b; it was not detected in the other samples, such as those fractionated after 23 min. As described above, we have

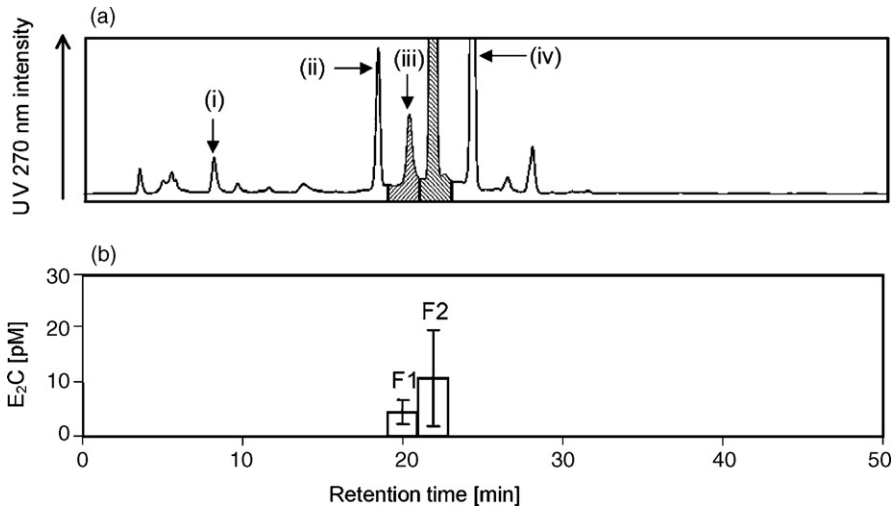


Fig. 5. HPLC fractionation and the value of E_2C. (a) A chromatogram for the irradiated solution during 160 min. (i) MMS, (ii) fenthion sulfoxide, (iii) MMP and (iv) fenthion. (b) E_2C of each fractionated sample in the present assay. Each bar represents the mean ± S.D. ($n = 3$).

already investigated the estrogenic activity of the detected compounds such as MMS (Fig. 5a(i)), fenthion sulfoxide (Fig. 5a(ii)), MMP (Fig. 5a(iii)), and fenthion itself (Fig. 5a(iv)), and we have also observed their HPLC retention times. It was found that the retention time of MMP was 20.1 min, and this completely corresponded with the value for F1. Therefore, we judged that the activity illustrated as F1 was caused by MMP. On the other hand, we could not identify the compounds illustrated as F2 from the retention time. To perform various analyses for F2, we periodically re-fractionated the solution irradiated during 160 min to obtain a large amount of F2, and accumulated the fractions obtained from 21 min to 23 min (near the value for F2) by using HPLC. From the HR FAB/MS measurements, we speculated that the molecular weight of the unidentified compounds was 279.0284 and the molecular formula was $C_{10}H_{15}O_3PS_2$. This formula completely corresponds to that of fenthion itself, but it should be pointed out that the estrogenic activity of fenthion was little detected in the present assay; moreover, its retention time for HPLC was 24.2 min, which is significantly greater than that of F2 (21 min to 23 min). These findings indicate that the unidentified compound must be an isomer of fenthion. Hence, we performed various NMR measurements, such as ^{1}H and ^{13}C NMR, nuclear overhauser experiments (NOE), ^{1}H detected multiple quantum coherence spectrum (HMQC), and ^{1}H detected multiple bond heteronuclear multiple quantum coherence spectrum (HMBC), to clarify the molecular structure. From the obtained NMR spectra, we speculated that the molecular structure was almost similar to that of fenthion, but the alternation of S-atom and O-atom in the molecule takes place, as shown in the left-side in Fig. 6. In the present study, we refer to the new compound as S-aryl fenthion. Detailed NMR spectra and FAB/MS data are as follows: ^{1}H NMR (methanol-*d*, TMS): δ 7.41 (d, 1H, $J = 8.2$, H-6), 7.35 (s, 1H, H-2), 7.21 (d, 1H, $J = 8.1$, H-5), 3.81 (d, 6H, $J = 12.6$, H-9), 2.45 (s, 3H, H-8), 2.28 (s, 3H, H-7); ^{13}C NMR: δ 142.4 (C-4), 137.8 (C-3), 136.8 (C-2), 134.2 (C-6), 125.8 (C-5), 120.9 (C-1), 55.3 (C-9), 19.8 (C-

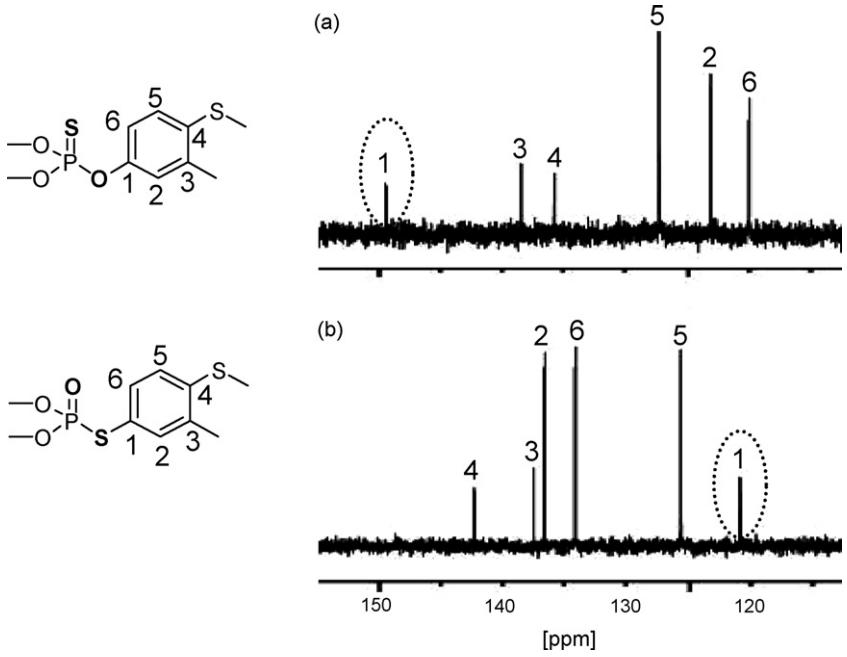


Fig. 6. ^{13}C NMR spectra of fenthion (a) and S-aryl fenthion (b). The molecular structure is also illustrated in the left-hand side in each figure, respectively. ^{13}C signals were assigned to the number described in the molecular structure.

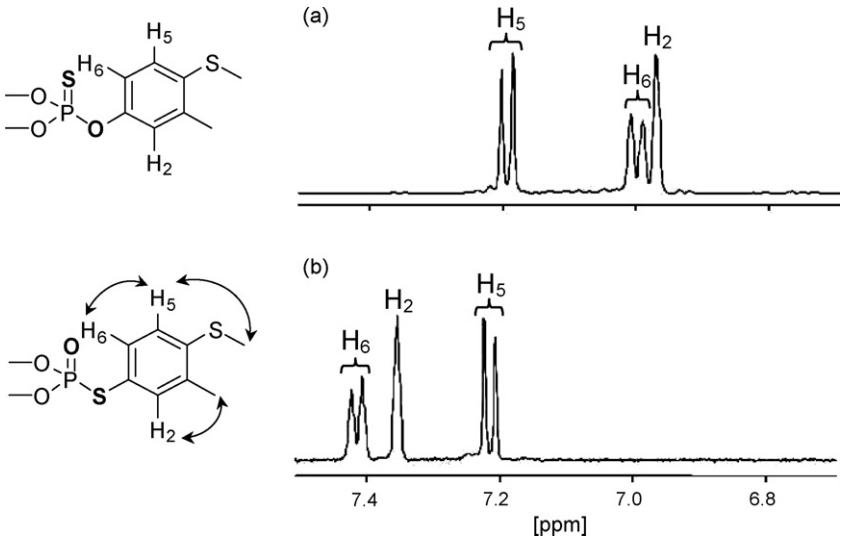


Fig. 7. ^{1}H NMR spectra of fenthion (a) and S-aryl fenthion (b). The molecular structure is also illustrated in the left-hand of each figure. ^{1}H signals were assigned to the number described in the molecular structure. The assignments are described in detail in Section 3.3. The arrows in this figure show NOE correlations.

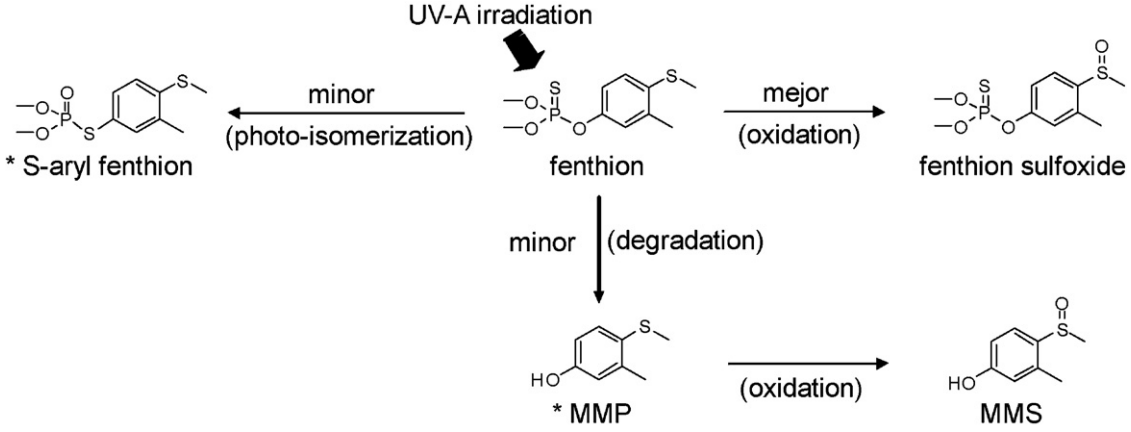


Fig. 8. Proposed scheme for the degradation of fenthion by UV-A irradiation. The asterisk (*) indicates that the compounds were regarded as estrogenic active compounds in the present assay.

7), 14.6 (C-8); HR FAB + MS *m/z* $(M+H)^{+}$: 279.0284 (intensity, 100%; calculated for 279.0278, $C_{10}H_{16}O_3PS_2$). To support this speculation, we investigated the difference in the molecular structure characteristics of S-aryl fenthion and fenthion by ^{13}C NMR measurements and quantum chemical calculations. The right-side in Fig. 6 shows the ^{13}C NMR spectra. It should be noted that the signal shown as "1" in Fig. 6a significantly shifted upfield in Fig. 6b, which is illustrated as a dotted circle; the value was 28.7 ppm. In addition, the signals shown as "2" and "6" in Fig. 6a slightly shifted downfield in Fig. 6b. On the other hand, the magnetic shielding tensors (ppm) were calculated by using the Gaussian 98 Rev. A. software program package. It was found that a similar shift in signal "1" illustrated in Fig. 6 was also observed in terms of the calculated tensors. It is well-known that the electron shield effects around a nucleus depend not only on electronegativity but also on resonance effects. Therefore, the shift calculated from the quantum chemistry also reflects the alternation of the S-atom and O-atom in the molecule; in other words, the calculation results supported the shift obtained from ^{13}C NMR measurements. Furthermore, Nichol and Elsbury [28] investigated the impurities included in commercial Diazinon, which is well-known as one of the most useful OPI. They also found a shift in a part of the signals in ^{1}H NMR spectra between diazinon and diazinon isomer, the so-called S-aryl diazinon, and they discussed the shift in terms of the alteration of S-atom and O-atom in the diazinon molecule. By comparing this with the shifts in the signals in ^{1}H NMR, the tendency, for example, that of signals "$H_{2''}$ and "$H_{6''}$ to shift downfield with the transformation to S-aryl fenthion, completely corresponded with that reported by Nichol and Elsbury as shown in Fig. 7. From these results, we finally concluded that the unidentified estrogenic active compound was S-aryl fenthion. As given in Fig. 8, we have summarized the degradation and transformation schemes under the UV-A irradiation and their estrogenic activities. In last decade, many studies have analyzed and investigated the residue of OPIs in various foods or environmental samples such as surface river water [9,29–34]. In the present study, we have obtained significant information on the importance of estrogenic activity of not only insecticides but also degraded and/or transformed products. In our next research, we will synthesize S-aryl fenthion and attempt to reveal its persistence or accumulation in various environmental samples.

4. Conclusion

We subjected fenthion to UV-A irradiation and evaluated its degradation rate constant as 0.0055 min^{-1} and its half-life as 126 min. Fenthion sulfoxide and MMS were identified as the major products and MMP was identified as the minor product. From the results of yeast two-hybrid assay, we found that the estro-

genic activity was not from fenthion itself; however, we detected significant activity in the UVA-irradiated solution. From investigations of the irradiated solution, we first ascertained the active compound to be MMP; however, from the contribution percentages of the estrogenic activity, we also noted that other active compounds were present in the solution. From our subsequent investigations, we found a new and potentially significant estrogenic active compound, S-aryl fenthion, which was induced by the photo-irradiation. From our results, we believe that not only the insecticide but also degraded or transformed products including its isomers must be quantitatively evaluated in various environmental media in order to estimate their impact on our health or ecological systems.

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