from which the amount of antibiotics present may be roughly estimated. Further determination of the exact quantity can then be easily carried out via the official potency assays.

From these overall features including the qualitative resolving power, semiquantitative estimation, feasibility for handling large number of samples in a single procedure, and sensitivity at the residual antibiotic levels in foods, it appears that AGE/AS method stands for a choice for detecting residual antibiotics in foods.

Seriousness of the problem concerning residual antibiotics in Taiwan area have been suggested by some research and survey activities. Thus Tsen *et al.* reported in 1974 that 17.12% of fresh milk in Taipei area showed positive reaction in triphenyltetraazonium chloride test[2]. Recent survey carried out in our laboratory, employing the CNS method, also indicated incidences as high as 30% in Taiwan area (unpublished data). While taking into consideration the defect inherited in these methods that these data may not be truly representative of the rate of residual antibiotics, a qualitative and quantitative survey system employing methods such as the AGE/AS technique is urgently needed for confirmation and for further tracing study.

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寒天凝膠電氣泳動及抗菌譜之抗生素系統分析法—用以檢驗食品中殘留抗生素之可能性

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摘　要

原由本實驗室發展之寒天凝膠電氣泳動及抗菌譜之抗生素系統分析法，經改良以增進其定性效果及敏感度，供試菌種增加爲6種後已可區分不同類之抗生素，寒天凝膠之厚度，供試菌種帶之厚度，及樣品縫之寬度等之最佳條件經定出而使本分析法之敏感度達到食品中抗生素殘留量之範圍，加上一次操作可處理多數量檢體之可行性及再顯性等條件一起評價，顯示利用本分析法以檢驗食品中殘留抗生素之可能性。

EFFECTS OF PADDY WATER AND SOME PHOTOSENSITIZERS ON THE PHOTOLYSIS OF THE FUNGICIDE ISOPROTHIOLANE

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(本文曾發表於加拿大環境健康科學雜誌 B15(2)，135-146，1980)

KEY WORDS: paddy water, riboflavin, oxygen, dithiolanylidenemalonate

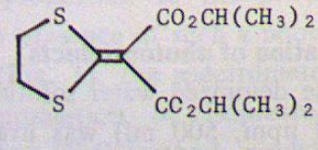
ABSTRACT

The fungicide isoprothiolane (diisopropyl 1,3-dithiolan-2-ylidenemalonate) decomposed slowly in deionized water under ultraviolet light or sunlight irradiation. Rice-paddy water greatly accelerated the photodegradation. This photosensitizing effect was comparable to that of 2% acetone. Soil extracts, rice-plant extracts, and chlorophylls showed little effect for the isoprothiolane photolysis. Tryptophan showed a relatively weak accelerating effect after a considerable lag time. Riboflavin exerted a remarkable acceleration of the photolysis. This effect was suppressed by a nitrogen gas stream.

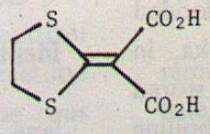
INTRODUCTION

The new systemic fungicide isoprothiolane (diisopropyl 1,3-dithiolan-2-ylidenemalonate) (I) is useful to control rice blast disease caused by *Pyricularia oryzae*.[1,2] It shows also a control effect against rice stem-rot (*Helminthosporium sigmoideum*). It is interesting to note that isoprothiolane has an effect to control certain insects, particularly planthoppers, too.[2] Isoprothiolane can be applied to paddy water as well as to foliage.

Photoreactions caused by solar light energy are of great importance for the fate of pesticides in the environment. The reactions are influenced by the conditions including the wavelength of light, the state of the substrate, and coexisting materials especially air, water, and sensitizers. In a previous short communication[3] we reported that by irradiation with short wave ultraviolet light isoprothiolane deposited on the silica gel thin layer underwent photoreactions via two different types of pathways; the degradation of the ester groups to give hydrolysis and decarboxylation products (II, III) and the transformation of the dithiolane ring into dithietane (IV) and trithiolane (V). The final product was S_8. This paper describes the photolysis of isoprothiolane in aqueous media and the effect of some photosensitizers. The photolysis was accelerated by paddy water and riboflavin in the presence of air.



isoprothiolane (I)



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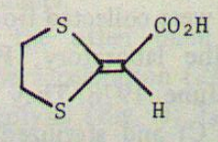

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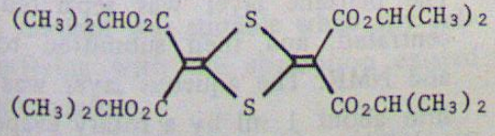

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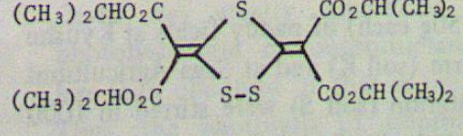

(V)

MATERIALS AND METHODS

Chemicals

Technical isoprothiolane (96%) was provided by Nihon Noyaku Co., Ltd. and purified by repeating recrystallization from *n*-hexane to get crystalline powder with a melting point of 57°C. It was homogeneous on the basis of thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and high pressure liquid chromatography (HPLC) and was used throughout this study. The authentic sample of 1,3-dithiolane-2-ylidenemalonic acid was a gift from Nihon Noyaku Co. All solvents used were redistilled in glass and all commercial chemicals were of first reagent grade quality.

A mixture of chlorophyll α and β was prepared from feces of silkworms by extraction with a mixture of petroleum benzine, benzene, and methanol and purified through a column of aluminacalcium carbonate-sucrose mixture.

Rice plant extracts

Air-dried rice straw (80 g) was soaked in 500 ml methanol for 5 days with occasional shaking at room temperature. Air-dried rice roots (50 g) were sliced into small pieces and extracted with 200 ml refluxing methanol for 24 hours. These extracts were filtered and concentrated to dryness by a rotary evaporator. The residues were used for photochemical experiments without purification.

Paddy water

Water samples were collected from a flooded paddy field near the laboratory, Fukuoka, in October, 1977 and June, 1978. They were stored in a cold-room (4°C) and sterilized by boiling and filtered before use.

Soil extract solutions

Soils (750g each) of paddy fields at Kyushu University Farm (soil K) and at Saga Agricultural Experiment Station (soil S) were stirred in 1000 ml deionized water for 24 hours. The supernatants were filtered and sterilized.

Photochemical procedures

Isoprothiolane was dissolved in an aqueous medium to a concentration of 20 or 50 ppm by stirring at 30°C. The solution (450 ml) was irradiated by a mercury lamp or sunlight in the presence or absence of additional chemicals or extracts. For indoor experiments, a photoreactor Riko UVL-100H-700P equipped with a 100W high pressure mercury lamp immersed in a Pyrex well was used. Emission of this lamp was mainly at 3126-3132, 3650-3663, 5461, and 5770-5791 nm. During irradiation, the reaction mixture was stirred with open to the atmosphere or under a nitrogen gas stream at 25°C.

Unreacted isoprothiolane was periodically determined by GLC, HPLC, or spectrophotometry. For the GLC analysis, 100 ml of the reaction mixture was withdrawn and extracted three times with 100 ml of benzene. The extracts dried on anhydrous sodium sulfate were concentrated into 0.5 ml by a rotary evaporator followed by a jet of nitrogen gas and submitted to GLC. For the HPLC or photometric determination, the 7 μl or 10 ml aliquot removed from the reaction mixture was directly submitted to HPLC or a spectrophotometer, respectively.

For sunlight irradiation, the aqueous isoprothiolane solution was placed in a 1500 ml plastic flat bath and was exposed to sunlight between 9 AM and 6 PM in fine July to August days (Fukuoka, Japan). Deionized water was supplied daily to make up for the loss of water by evaporation. Unreacted isoprothiolane was determined as mentioned above.

Identification of photoproducts

The deionized water solution of isoprothiolane (50 ppm, 500 ml) was irradiated with UV in the photoreactor for 105 hours and then extracted three times with 250 ml of benzene. The benzene layer was separated, dried, concentrated, and then submitted to GLC, TLC, and NMR. The aqueous layer was concentrated into about 1 ml by a rotary evaporator. A precipitate which appeared during concentration procedre was removed by filtration. The filtrate

was submitted to HPLC or TLC directly and also after methylation with diazomethane.

Other methods

UV spectrometic measurements were carried out on a Shimadzu UV-200 double beam spectrophotometer. A JEOL JGC-750-1100 gas chromatograph equipped with a flame ionization detector was used for the analysis of isoprothiolane. A stainless steel column (0.30 cm x 2.25 m) packed with 5% silicone SE-30 on 80/100 mesh Uniport KS was used for the analysis. Further confirmation was made on a glass column (0.3 cm x 2.25 m) packed with 15% silicone DC-550 on 60/80 mesh Uniport B. A Shimadzu 830 high pressure liquid chromatograph fitted with an ultraviolet photometric detector was used for the analysis of the water soluble photoproducts. An ODS Permaphase column and distilled water as the mobile phase were applied at 20 kg/cm^2. Proton nuclear magnetic resonance spectra were recorded on a JEOL NMR spectrometer MH-100 (100 MHz).

RESULTS AND DISCUSSION

The recovery of isoprothiolane from aqueous solution between 20 and 50 ppm by benzene extraction followed by GLC determination was 82%. No peak other than that of isoprothiolane was found on GLC determination of the benzene extracts of the photoreaction mixture in deionized water. Since isoprothiolane has characteristic maximium UV absorption at 312 nm, which decreased in proportion to the photolysis proceeding in the presence of such a photosenzitizer as riboflavin (Fig. 1), the spectrometric method for the determination of isoprothiolane was more useful than GLC method, provided neither coexistent materials nor photolysis products interfered with the measurement of the absorption. HPLC method was suitable when any substances interfering with the absorption existed in the reaction mixture.

Isoprothiolane decomposed slowly in deionized water by light irradiation. The half-life was 73.6 and 186 hours respectively, under irradiation with a high pressure mercury lamp and with sunlight. One sample of rice paddy water (1) greatly accelerated the photolysis rate of isoprothiolane (Fig. 2 and Table 1) The half-life shortened to 6.3 hours under UV irradiation. The effect of paddy water was almost comparable to that of 2% acetone, a wellknown triplet photosensitizer. The similar accelerating effect of paddy water has been reported in the photolysis of other pesticides.[4-6] The effect of paddy water 1 decreased, however, after storage for six months. Moreover, another sample of paddy water (2) exerted only a little effect on the isoprothiolane photolysis (Fig. 2; Table 1). These facts suggest that certain unstable photosensitizers are contained in paddy water, but their kind and concentration may vary according to the sampling place and time.

Riboflavin, chlorophylls, and other plant products may be natural photosensitizers possibly responsible for the environmental photodegradation of pesticides.[7,8] Paddy soil extracts (Fig. 2) and rice-plant extracts rather retarded the photolysis rate of isoprothiolane. Chlorphyll showed an evident but weak effect for the photolysis (Table 1; Fig. 4). Tryptophan, which had been suggested as a photosensitizer in paddy water by Crosby and his coworkers,[5] showed an interesting behavior during isoprothiolane photolysis (Fig. 4); the absorption at 312 nm increased at first and the photolysis rate (1.9 x 10^{-2} hr^{-1}) became about twice after 10 hr irradiation. These facts suggest that the photosensitizing activity of tryptophan may be due to its certain photoproducts.

On the other hand, riboflavin exerted a remarkable acceleration (24 times) of isoprothiolane photolysis (Fig. 4; Table 1). Similar effect of the chemical was also observed under sunlight (Fig. 3; Table 1). Under a nitrogen gas stream, however, the photolysis acceleration effect of riboflavin was greatly suppressed (Fig. 4). The photolysis rate of isoprothiolane reduced into less than one-third of that in the presence of air. Thus, the photolysis of isoprothiolane in the presence of the sensitizer and oxygen may be mainly due to photooxidation. Under sunlight

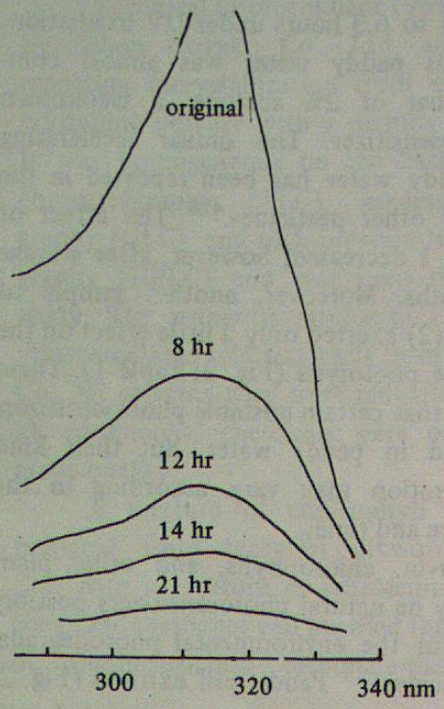

Fig. 1
UV spectra of isoprothiolane decomposing under sunlight in water containing 5 ppm riboflavin.

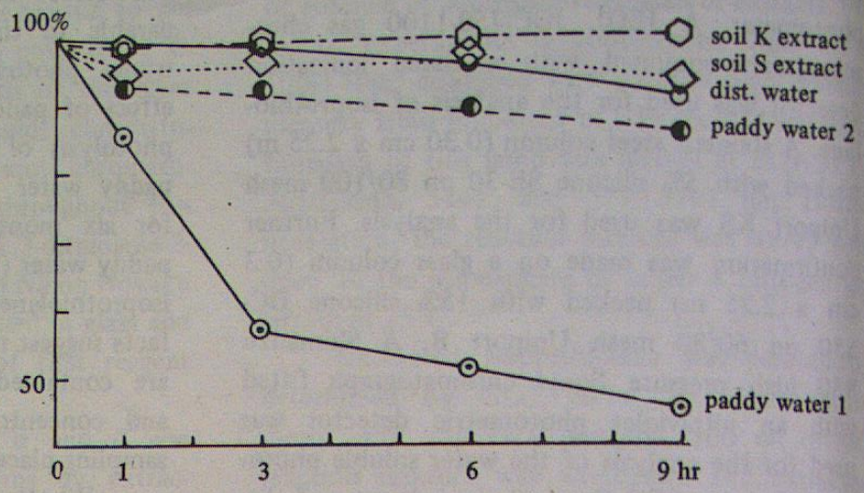

Fig. 2 Photolysis of isoprothiolane with UV in water

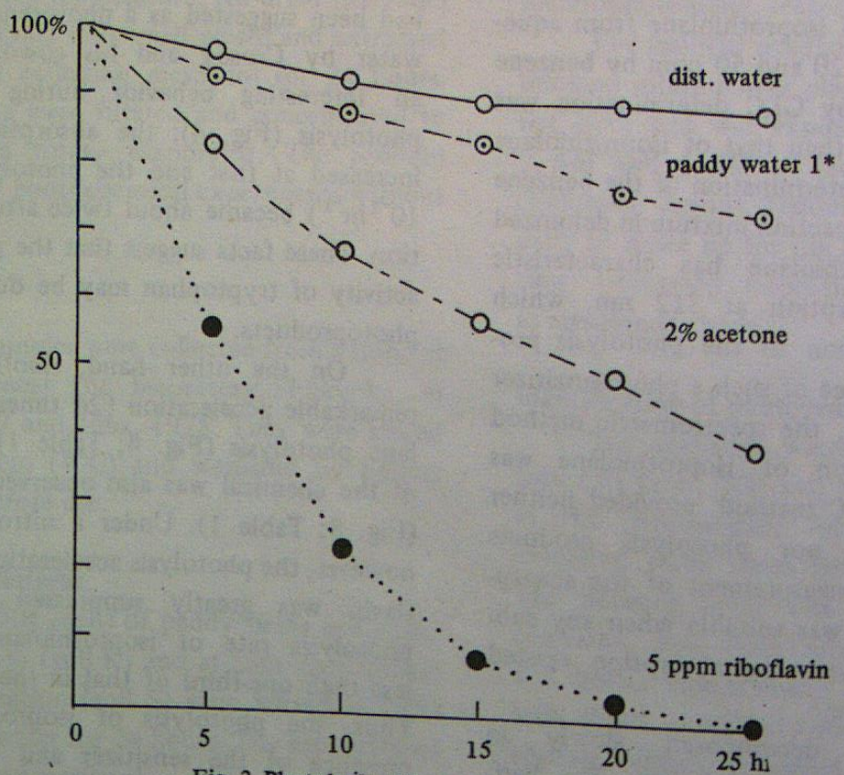

Fig. 3 Photolysis of isoprothiolane by sunlight
* stored for six months

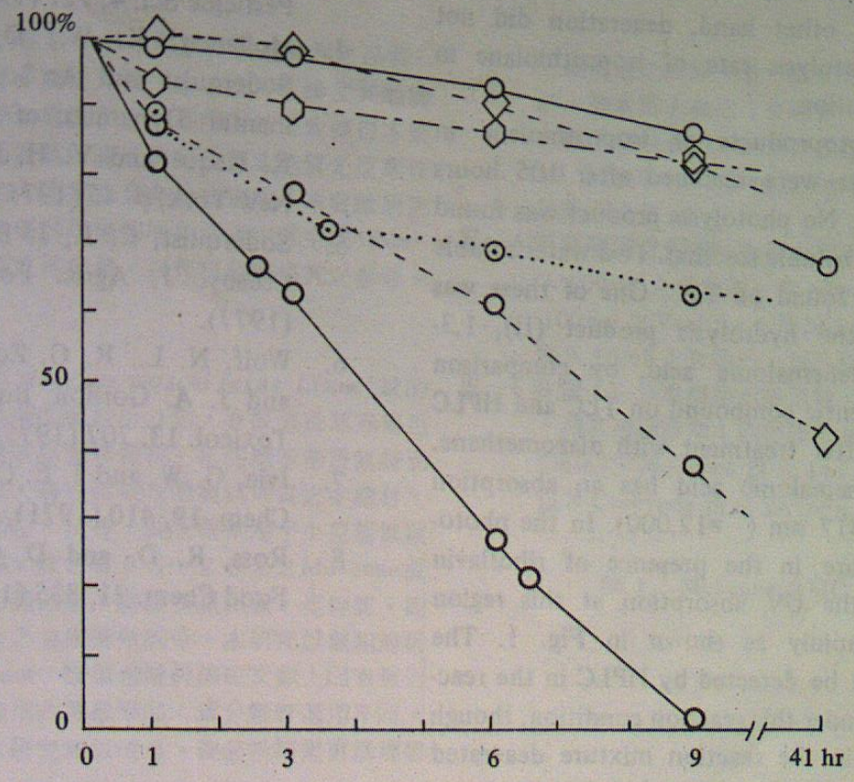

Fig. 4 Effect of photosensitizers on photolysis of isoprothiolane with UV.
—○— dist. water; - -◇- - tryptophan 50 ppm;
— . - chlorophyll 50 ppm; - -○- - acetone 2%;
. .○··· riboflavin/N_2 5 ppm; —●— riboflavin/air 5ppm

Table 1. Photolysis rate of isoprothiolane in aqueous solutions

Isoprothiolane solution	UV light		Sunlight	
	k ($\times 10^{-2} hr^{-1}$)	$t_{1/2}$ (hr)	k ($\times 10^{-2} hr^{-1}$)	$t_{1/2}$ (hr)
Dist. water	0.94	73.6	0.37	186.1
Paddy water 1	11	6.3	0.9*	75.3*
Paddy water 2	2.3	30.1		
Acetone, 2%	9.2	7.5	3.7	18.9
Chlorophyll, 50ppm	1.4	49.2		
Riboflavin, 5ppm	23	3.0	15.9	4.4

*Paddy water 1 stored for six months was used.