Photodecomposition of Endosulfan and Related Products in Thin Films by Ultraviolet Light Irradiation

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Ultraviolet irradiation of endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) isomers I and II in a thin film produced endosulfandiol as a major product. Endosulfan α-hydroxy ether, lactone, ether, and an unknown No. 1 were additional photodecomposition products but in lesser amounts than the diol. Irradiation of endosulfandiol produced α-hydroxy ether, unknowns No. 2 and 3. Irradiation of endosulfan ether gave the α-hydroxy ether and the lactone. Irradiation of endosulfan α-hydroxy ether gave the ether and unknowns No. 2 and 3. Irradiation of endosulfan lactone resulted in less than 1% each of the diol and the ether, but no α-hydroxy ether was produced. The endosulfan sulfate was not a product from any of the irradiated compounds, and no degradation products were detected when it was irradiated with ultraviolet light. Derivatization of the diol and the α-hydroxy ether by silylation and acetylation was necessary for the quantitation and identification of these two compounds within the photolysis mixtures.

Photolysis of pesticides is important in the fate of these compounds in the environment and affects their toxicological characteristics. For example, Roburn (1963) found that grasses sprayed with dieldrin and exposed to sunlight under natural conditions for several months contained an unknown persistent compound. Dieldrin deposited as a film on glass plates and irradiated under a germicidal ultraviolet lamp (2537 Å) was converted to some similar persistent compounds found on the grasses. The fact that strong artificial ultraviolet radiation can cause chemical changes of chlorinated pesticides has been observed (Mitchell, 1961, 1962; Harrison *et al.*, 1967; Henderson and Crosby, 1968).

Thiodan, known also as endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide), is classified among the chlorinated hydrocarbons of the cyclodiene group. It is a mixture of two isomers I and II, which are in the approximate ratio of 70:30%, respectively.

The fate of Thiodan on plant surfaces and in the laboratory has been studied by several investigators. Lindquist and Dahm (1957) reported that endosulfan was hydrolyzed on plant foliage to endosulfan diol. Terranova and Ware (1963), however, could only find small amounts of endosulfan ether plus the two isomers of the insecticide (but no diol) on the leaves of *Vicia faba* (broad beans) 1, 2, 3, and 4 weeks after spraying the plants with endosulfan solution in acetone. The fate of endosulfan isomer I and II under controlled and greenhouse conditions was investigated by Ware *et al.* (1967–1968). The sulfate was the only metabolite detected in the greenhouse. Volatile metabolites found under controlled conditions were the sulfate and the ether. Endosulfan sulfate was obtained as a residue component on leafy vegetables sprayed with endosulfan (Cassil and Drummond, 1965). They hypothesized that the mechanism for the oxidation of endosulfan was by active oxygen derived from growing plant tissue. All attempts to obtain endosulfan sulfate from a thin film of endosulfan on a glass plate in the presence of air and oxygen at different temperatures failed.

Harrison *et al.* (1967) found that the exposure of endosulfan dissolved in liquid paraffin to ultraviolet irradiation in the laboratory did not produce any distinguishable different compounds, but some endosulfan sulfate was formed after irradiation of technical endosulfan dissolved in aqueous glycerol.

The aim of these investigations is to study the photodecomposition products of endosulfan-related compounds under controlled laboratory conditions using tlc–glc with silylation and acetylation procedures for the identification of the photodecomposition products and to express the results at least semiquantitatively.

MATERIALS AND METHODS

Chemicals. All standards were obtained from the Niagara Chemical Division, FMC Corp., Middleport, N.Y., except endosulfan α-hydroxy ether and lactone, which were provided by G. W. Ware, Department of Entomology, University of Arizona, Tucson, Ariz. Isomer I was composed of 67.00% isomer I, 30.00% isomer II, 2.40% diol, and 0.60% ether. Isomer II was composed of 8.20% isomer I, 83.70% isomer II, 5.30% diol, and 2.80% ether. All other standards were essentially 100% pure. Figure 1 contains three-dimensional molecular structures of endosulfan and related compounds.

Irradiation. Two-hundred milligrams of each standard was dissolved in 20 ml of acetone and spread in a thin film in a 20 × 20 × 5 cm borosilicate glass baking dish by allowing the acetone to evaporate. The sample was irradiated with an ultraviolet lamp containing two 15-W, 35.56-cm effective length, 2.54-cm diameter, General Electric germicidal lamps (G15T8) at a distance of 6 cm above the thin layer for 7 days. Temperatures ranged from 30 to 35°. Ultraviolet light intensity was measured with a Black-Ray Ultraviolet Intensity Meter (Ultra-Violet Products, Inc., Model J227 metering unit, J226 short wavelength cell) measuring microwatts per square centimeter, with an accuracy of ±5% and converted to ergs per square centimeter intensity. Ultraviolet light exposure during the experiments was at approximately 1×10^4 ergs per cm^2.

Dark Experiments. Thin films of endosulfan isomers I and II and sulfate covered with cheesecloth were allowed to remain in a ventilated hood not exposed to ultraviolet light to determine the effects that would occur without light exposure but with air exposure.

Identification of Photolysis Products. THIN-LAYER CHROMATOGRAPHY (tlc). Tlc was conducted on 20 × 20 cm glass plates coated with 0.25-mm thickness of silica gel H, de-

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Table I. Thiodan and Related Compounds Properties after No Treatment, Silylation, and Acetylation as Obtained by tlc and glc

Compound	Empirical formula	tlc[a] R_f values			glc[b] R_t values		
		No treatment	Silylated	Acetylated	No treatment	Silylated	Acetylated
Endosulfan isomer I	$C_9H_6O_3Cl_6S$	0.52	0.52	0.52	5.6	5.6	5.6
Endosulfan isomer II	$C_9H_6O_3Cl_6S$	0.30	0.30	0.30	9.5	9.5	9.5
Endosulfandiol	$C_9H_8O_2Cl_6$	0.14	0.14	0.28	3.9[c]	5.3	9.8
					10.0[d]		
Endosulfan ether	$C_9H_6O_4Cl_6$	0.41	0.41	0.41	2.3	2.3	2.3
Endosulfan sulfate	$C_9H_6O_4Cl_6S$	0.21	0.21	0.21	13.3	13.3	13.3
Endosulfan α-hydroxy ether	$C_9H_6O_2Cl_6S$	0.21	0.21	0.21	3.1[e]	3.1	5.1
					4.0[f]	4.0	
Endosulfan lactone	$C_9H_4O_2Cl_6$	0.21	0.21	0.21	5.5	5.5	5.5

[a] Tlc signifies thin-layer chromatography. [b] Glc signifies gas–liquid chromatography. [c] First peak of endosulfandiol. [d] Second peak of endosulfandiol. [e] First peak of endosulfan α-hydroxy ether. [f] Second peak of endosulfan α-hydroxy ether.

veloping solvent *n*-hexane–acetone 6:1 (v/v), and the silver nitrate–2-phenoxyethanol color test (Mitchell, 1958) as a dip solution were employed.

Tlc was employed for screening and in combination with glc, as an analytical tool. For quantitative work, chromatogram areas containing the unknowns were extracted from the silica gel with benzene–isopropyl alcohol (1v/1v), after comparing R_f values with those of parallel standards.

GAS–LIQUID CHROMATOGRAPHY (glc). Glc was accomplished with: a Varian Aerograph, Model 1200 gas chromatograph equipped with an electron capture detector; a Texas Instrument Co. model Servo/Riter II recorder (1 mV) with a chart speed of $^1/_2$ in. per min; and an 8 ft by $^1/_8$ in. o.d. stainless steel column packed with 5% SE30 + 5% DC710 on Chromosorb W (60/80 mesh), acid washed, and treated with DMCS. The temperature of the injection port was 225°; detector was 195°; column was 195°; and it had a nitrogen carrier gas flow of 20 ml/min.

SILYLATION. Five milligrams of the standards, either irradiated or nonirradiated, were placed in stoppered tubes. Regisil [bis(trimethylsilyl)trifluoro acetamide], 0.15 ml, was added to the standards and the volume was adjusted to 5 ml with benzene–isopropyl alcohol (1v/1v). The solution was well mixed and placed in a preheated water bath at 60° for 1 hr. The sample was removed from the bath, cooled, and analyzed by glc and tlc (Ware *et al.*, 1967–1968).

ACETYLATION. One-hundred micrograms each of the endosulfan isomer I, isomer II, diol, ether, α-hydroxy ether, lactone, and sulfate, either irradiated or nonirradiated, were acetylated according to the method of Chau (1969) with the exception that the acetylation reagent and the sodium carbonate were doubled in amount during the acetylation treatment.

RESULTS AND DISCUSSION

Tlc was a very useful tool in the identification of the endosulfan photodecomposition products. Endosulfan α-hydroxy ether, lactone, and sulfate have identical R_f values as shown in Table I. Silylation of these standards did not improve their separation on tlc. Acetylation increased the R_f of the diol, however.

Analysis of the standards on glc under the stated conditions gave one peak, except that both the diol and the α-hydroxy ether gave two peaks. Glc showed overlap between isomer I and the lactone, and overlap between the first peak of the diol and the second peak of the α-hydroxy ether. Silylation resolved the diol from the α-hydroxy ether when analyzed by glc (Table I), and the R_t of the diol was increased to that of the lactone leaving the α-hydroxy ether without interference. Acetylation increased the R_f of the diol so it could be determined without interference.

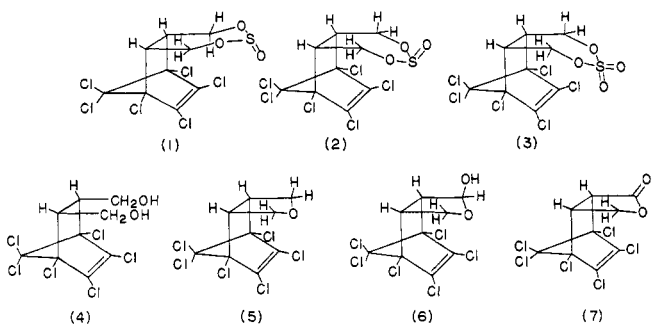

Figure 1. Three-dimensional diagrams of the chemical structures of (1) endosulfan, isomer I, (2) endosulfan, isomer II, (3) endosulfan sulfate, (4) endosulfandiol, (5) endosulfan ether, (6) endosulfan α-hydroxy ether, and (7) endosulfan lactone

The following procedure was followed in the quantitation of the standards or the photodecomposition products. Tlc was employed as the first step in the separation of the photolysis products. Irradiated samples and standards were spotted side by side and the tlc plates were solvent developed. The chromatogram areas containing the standards and the unknowns were extracted from the silica gel after comparing the R_f values with those of parallel standards visualized by chromogenic development. Extraction recoveries of the compounds from the tlc plates ranged from 80 to 102%. The standards were extracted parallel with the unknowns so that the recovery of the standards and the photoproducts would be relative.

The chromatogram areas of isomers I and II and the ether were completely separated. The chromatogram areas of the diol, α-hydroxy ether, lactone, and sulfate were not completely resolved as shown by the R_f values in Table I.

The combination of the tlc–glc procedures made it possible to determine endosulfan isomer I, isomer II, ether, lactone, and the sulfate without interferences on the glc, but not the diol and the α-hydroxy ether since they overlapped on the glc. Silylation of the tlc chromatogram areas which contained the diol and the α-hydroxy ether allowed glc analysis without interference (Table I) since the R_t of the diol was increased and the R_t of the α-hydroxy ether remained unchanged. The diol, after silylation, however, overlapped with the lactone and interfered with the diol determination.

Acetylation of the tlc chromatogram areas which contained the diol and the α-hydroxy ether provided separation

Table II. Percentages of Components in the Endosulfan-Related Standards after Ultraviolet Light Irradiation

Endosulfan	I	II	Diol	Ether	α-Hydroxy ether	Lactone	Sulfate
	Percent of original compound lost by volatilization						
	43.32	55.40	72.45	81.25	74.50	65.59	69.80
	Percent conversion to following products expressed as original standard						
I	57.60	3.20	N.D.[a]	N.D.	N.D.	N.D.	N.D.
II	8.45	45.00	N.D.	N.D.	N.D.	N.D.	N.D.
Diol	21.91	29.70	52.60	0.56	3.70	0.56	N.D.
Ether	1.76	0.94	0.24	35.40	18.80	0.70	N.D.
α-Hydroxy ether	2.18	8.50	25.20	31.80	47.60	N.D.	N.D.
Lactone	2.58	1.50	2.76	30.00	11.80	96.50	N.D.
Sulfate	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	30.20
Unknown 1	1.34	2.90	N.D.	N.D.	N.D.	N.D.	N.D.
Unknown 2	N.D.	N.D.	7.80	0.67	15.00	2.03	N.D.
Unknown 3	N.D.	N.D.	11.40	0.51	2.60	N.D.	N.D.

[a] N.D. signifies nondetectable.

of the diol and the lactone by glc. The diol R_t after acetylation was increased to 9.8 min and the lactone R_t remained at 5.5 min (Table I).

Table II shows the percentages of the components in the endosulfan-related standards after their exposure to ultraviolet light.

The α-hydroxy ether and the lactone which have not previously been detected as decomposition products on plant surfaces or glass plates exposed to ultraviolet light were detected as photodecomposition products in these investigations. Endosulfan isomer I, isomer II, diol, and ether all produced α-hydroxy ether (Table II) upon ultraviolet light irradiation. However, the α-hydroxy ether has been reported as a metabolite of endosulfan in rats (Ballschmiter and Tölg, 1968). Ballschmiter and Tölg (1966) detected endosulfan sulfate, ether, α-hydroxy ether, and lactone as transformation products of endosulfan in the migratory locust (*Pachytilus migratorius migratorioides*).

Endosulfan isomer I, isomer II, diol, α-hydroxy ether, and ether upon irradiation also produced lactone (Table II). The diol was the major product from the irradiation of isomers I and II (Table II). The sulfate was not detected as a photodecomposition product in any of the irradiated compounds, and no degradation products were produced when it was irradiated.

The conversion of endosulfan to the sulfate depends on the experimental conditions. Terranova and Ware (1963) did not find any sulfate on broad beans sprayed with endosulfan, but later Ware *et al.* (1967–1968) reported that the sulfate was the only metabolite detected under greenhouse conditions. Harrison *et al.* (1967) found endosulfan sulfate produced only when the endosulfan was dissolved in aqueous glycerol and was exposed to ultraviolet light.

In addition to the known photodecomposition products of endosulfan-related compounds, three unknown materials were detected in the ultraviolet light-treated compounds in the present investigations (Table II). The extraction of a tlc chromatogram area at R_f 0.41 usually produced the endosulfan ether. However, the irradiated sample from the tlc area R_f 0.41 upon glc analysis contained an unknown peak. The R_f of this unknown No. 1 did not match any of the known standards. It was resolved as a shoulder, R_t 5.3 min, on the isomer I peak with a R_t of 5.5 min.

Irradiated endosulfan diol, ether, α-hydroxy ether, and lactone produced two additional unknowns when analyzed by tlc–glc (Table II). The tlc R_f value for both unknowns was 0.19. Unknown No. 2 had a R_t of 3.6 min, and unknown No. 3 had a R_t of 8.6 min. These two unknown peaks did not match any of the known standards. The irradiated ether gave less than 1% of unknowns No. 2 and No. 3. The irradiated lactone contained 2% of the unknown No. 2 and no unknown No. 3.

The dark experiments (no ultraviolet irradiation) were considered as controls for the ultraviolet light experiments. The dark treatment of endosulfan isomer I, isomer II, and the sulfate resulted in no detectable degradation products.

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