small amounts in samples which have stood for several days or more.

The metallic dialkyldithiocarbamates have been shown to exhibit inhibitory effects on certain enzyme systems (Thorn and Ludwig, 1962; Dailey *et al.*, 1969). In addition, the results of this study indicate that in any determination of the biological effects of ethyl selenac, the presence of thiuram disulfide, selenite, and diethyldithiocarbamate must be considered. Selenite ion is known to inhibit several enzyme systems (Rosenfeld and Beath, 1964). TETD, also known as Antabuse or Disulfiram, is not only a rubber accelerator, vulcanizer, seed disinfectant, and fungicide *per se*, but also has antialcoholic utility and exhibits a battery of *in vivo* effects. Among the known biological effects of TETD are: inhibition of acetaldehyde dehydrogenase (Graham, 1951); prolongation of hexobarbitol sleeping time in rats (Maj and Przegalinski, 1967); inhibition of dopamine β-hydroxylase in rats (Goldstein *et al.*, 1964); increase in the toxicity of diphenylhydantoin in man (Olesen, 1966); and impairment of drug metabolism by rat liver microsomes (decrease in *N*-demethylase activity) (Stripp *et al.*, 1969). Additionally, diethyldithiocarbamate, an intermediate in the decomposition studied here and a metabolite of TETD, has been shown to have some inhibitory effect on dopamine β-hydroxylase (Musacchio *et al.*, 1964). Since in natural environmental conditions aqueous media are the rule, the pathway for production of diethyldithiocarbamate and its further degradation to diethylamine and CS_2 assumes perhaps even greater relevance than the production of TETD. Thus, to account for the known effects of ethyl selenac as well as to predict the effects of its breakdown in the environment, the presence of *N,N,N′,N′*-tetraethylthiuram disulfide, *N,N*-diethyldithiocarbamate, selenite, and elemental selenium as well as *their* breakdown products must be considered.

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Rotenone Photodecomposition

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Irradiation of rotenone in oxygenated methanol solution with ultraviolet light yields the following crystalline products: *O*-demethylrotenone; 6aβ,12aβ-rotenolone; rotenonone; and three acids (4,5-dimethoxysalicylic, rissic, and tubaic acids) which were methylated prior to their isolation. Rotenone photodecomposes on plant leaves exposed to sunlight and on glass surfaces irradiated with sunlight, ultraviolet light, or a sunlamp to yield at least 20 compounds, including *O*-demethylrotenone, 6′,7′-epoxyrotenone, 6aβ,12aβ- and 6aβ,12aα-rotenolones, 6′,7′-epoxy-6aβ,12aβ-rotenolone, 6a,12a-dehydrorotenone, and rotenonone. Methods of synthesis are described for the new *O*-demethyl- and 6′,7′-epoxy derivatives of rotenone. In the determination of residues in or on crops treated with rotenone, it is important to determine the major toxic photodecomposition product, 6aβ,12aβ-rotenolone, as well as rotenone.

Rotenone effectively controls many phytophagous insects but only for a short period of time after application because it decomposes in the presence of light and air. On the basis of work done several decades ago, it is known that rotenone photodecomposes in solution and as spray residues to yield rotenolones, 6a,12a-dehydrorotenone, rotenonone, and a mixture of unidentified products (Cahn *et al.*, 1945; Jones and Haller, 1931; Subba Rao and Pollard, 1951). More recent studies on rotenone chemistry serve as a basis for a reexamination of the mechanisms and pathways of rotenone photodecomposition (Crombie, 1963).

This paper describes the products obtained when rotenone solutions in methanol and benzene are exposed to ultraviolet (uv) light, while oxygen is bubbled through the solutions, and

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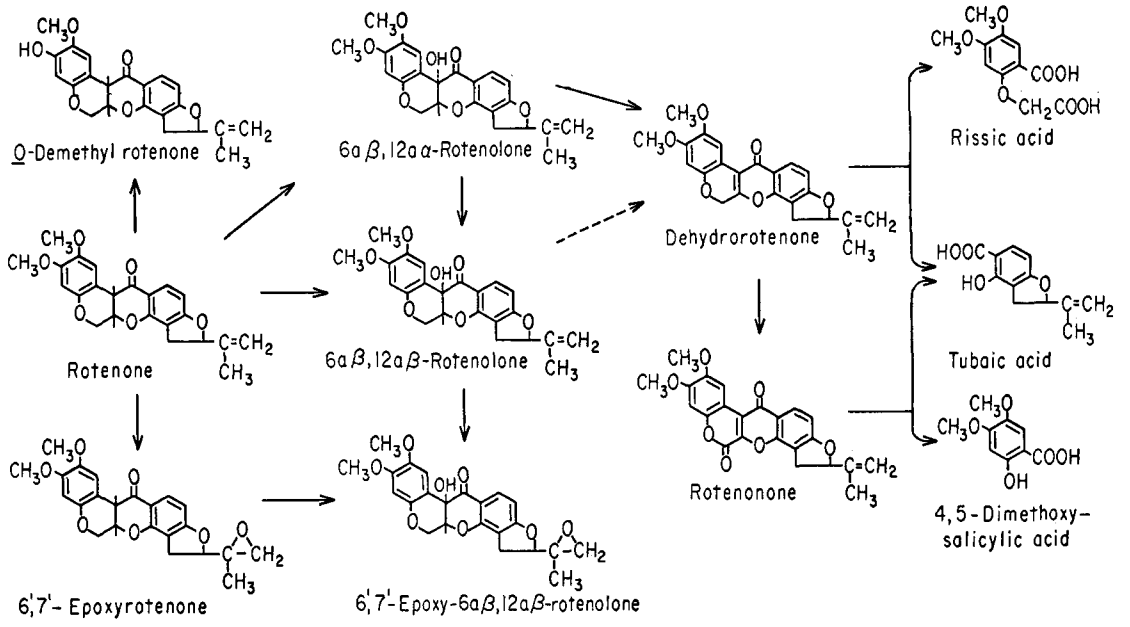

Figure 1. Partial pathway for photodecomposition of rotenone

when plant leaves or glass surfaces are treated with rotenone-6a-^{14}C and exposed to uv light, sunlamp irradiation, or sunlight.

MATERIALS AND ANALYTICAL METHODS

Chemicals. Natural rotenone (Aldrich Chemical Co., Milwaukee, Wis.) was recrystallized from methanol [mp 162–3°C, reported as 162–3°C by Nishizawa and Casida (1965), and 167–8°C by Crombie (1963); $[\alpha]^{25}D$ −226° in benzene, reported as $[\alpha]^{20}D$ −228° in benzene by Crombie (1963)]. Rotenone-6a-^{14}C was prepared by Nishizawa and Casida (1965).

A number of rotenone derivatives or degradation products were synthesized or obtained elsewhere. [The Roman numerals given below, after the names of some compounds, indicate that they are not structurally defined in Figure 1; the numerals given refer to the respective structures assigned to the compounds by Crombie (1963).] Rotenone was decomposed in ethanolic potassium hydroxide solution in the presence of zinc powder (Hamada and Chubachi, 1969; LaForge and Smith, 1929) to give derritol (XLII) [mp 162–3°C, reported as 164°C; mass spectra (ms) fragments at 370 (M^+), 203, 194, 175, 167, 166, 147, 137] and rotenol (LIX–LX) (mp 118–9°C, reported as 120°C). Treatment of rotenone with 5% ethanolic potassium hydroxide yielded tubaic acid [mp 127–8°C, reported as 129°C by Haller and LaForge (1930); ms fragments at 220 (M^+), 203, 202, 176, 174, 146] [methyl ester: mp 51–2°C, reported as 48°C by Butenandt (1928); ms fragments at 234 (M^+), 203, 202, 175, 174, 146; infrared (ir) spectral peaks at 3200–3100 (hydrogen-bonded OH), 1665 (C=O), 1625, 1600, and 1490 cm^{-1} (phenyl)]. Leslie Crombie (University of Nottingham, Nottingham, England) or Masanao Matsui (The University of Tokyo, Tokyo, Japan) provided the following compounds: 6a,12a-dehydrorotenone [ms fragments at 392 (M^+) and 377]; rotenonone [mp 286–8°C, reported as 298°C by LaForge (1932); ms fragments at 406 (M^+) and 391]; (−)-rotenol (LIX); (+)-6,6a- and (−)-6,6a-dehydrorotenols (IL and LI, respectively); 6a,12a-dehydrorotenol (Jennen phenol) (LIII); 6aα,5'β- and 6aβ,5'β-ketoaldehyde derivatives of rotenone (XC and LXXXIX, respectively); derric acid (XXXIX); derrisic acid (XXXVIII); rissic acid (mp 252–3°C, reported as 256°C by Takei *et al.*, 1931) [dimethyl ester: mp 83–4°C, reported as 86°C by Takei *et al.* (1931); ms fragments at 284 (M^+), 269, 253, 252, 225; uv spectrum (λ_{max}) 207, 223, 258, 304 mμ]; toxicaric acid (LXV).

4,5-Dimethoxysalicylic acid (mp 209–10°C, reported as 210–1°C by Rajagopalan *et al.*, 1949) was synthesized by three different routes involving the following respective intermediates: in the first route, resorcinol, β-resorcylic acid, 4-methoxysalicylic acid methyl ester, 4-methoxy-5-hydroxysalicylic acid, and 4,5-dimethoxysalicylic acid methyl ester [mp 94–5°C, reported as 95°C by Rajagopalan *et al.* (1949); ms fragments at 212 (M^+), 181, 180, 153, 152; uv spectra (λ_{max}) 209, 226, 262, 318 mμ]; in the second route, *p*-benzoquinone, 1,2,4-triacetyl hydroxyhydroquinone, 2-hydroxy-*p*-hydroquinone, 4,5-dihydroxysalicylic acid, and 4,5-dimethoxysalicylic acid methyl ester; in the third pathway, vanillin, veratric aldehyde, and 3,4-dimethoxyphenol. Each route gave the same final product [mp and thin-layer chromatography (tlc)].

Rotenolone isomers were obtained by recrystallization of the products from alkaline oxygenation of rotenone; one product was a mixture of the *trans*-rotenolone isomers (6aβ,-12aα and 6aα,12aβ) and the other was a mixture of the *cis*-rotenolone isomers (6aβ,12aβ and 6aα,12aα) (Crombie and Godin, 1961). (Byproducts of the alkaline oxygenation reaction are small amounts of 6a,12a-dehydrorotenone and rotenonone.) Each isomer in the *trans*-rotenolone mixture was isolated by preparative tlc using four sequential developments with carbon tetrachloride–ether mixture (4:1) and each isomer in the *cis*-rotenolone mixture was isolated using carbon tetrachloride–ether mixture (6:1) or hexane–ethyl acetate mixture (2:1), each developed several times, in sequence. [The characterization of these rotenolones is given by Unai *et al.* (1972).]

8'-Hydroxyrotenone, 6',7'-dihydro-6',7'-dihydroxyrotenone, the rotenolones derived from these two compounds, and 6',7'-epoxy-6aβ,12aβ-rotenolone were synthesized according to Unai *et al.* (1972).

Table I. Tlc Solvent Systems and R_f Values for Rotenone Derivatives

Compounds	R_f values in indicated solvent systems[a]			
	A	B	C	D
Rotenone	0.55	0.20	0.25	0.51
6a,12a-Dehydrorotenone	0.51	0.21	0.25	0.53
O-Demethylrotenone	0.37	0.13	0.15	0.39
6′,7′-Dihydro-6′,7′-dihydroxy-rotenone	0.07	0.01	<0.01	0.03
6′,7′-Dihydro-6′,7′-dihydroxy-6aβ,12aβ-rotenolone	0.03	0.01	<0.01	0.02
6′,7′-Dihydro-6′,7′-dihydroxy-6aβ,12aα-rotenolone	0.02	0.01	<0.01	0.02
6′,7′-Epoxyrotenone	0.48	0.08	0.12	0.39
6′,7′-Epoxy-6aβ,12aβ-rotenolone	0.37	0.07	0.10	0.30
8′-Hydroxyrotenone	0.21	0.03	0.03	0.16
8′-Hydroxy-6aβ,12aβ-rotenolone	0.14	0.03	0.03	0.12
8′-Hydroxy-6aβ,12aα-rotenolone	0.08	0.03	0.03	0.11
6aβ,12aβ-Rotenolone	0.42	0.15	0.18	0.41
6aα,12aα-Rotenolone	0.42	0.13	0.16	0.41
6aβ,12aα-Rotenolone	0.38	0.18	0.18	0.48
6aα,12aβ-Rotenolone	0.38	0.19	0.24	0.52
Rotenonone	0.56	0.21	0.25	0.54

[a] Composition of tlc solvent systems: A = benzene–methanol (30:1); B = hexane–ethyl acetate (2:1); C = carbon tetrachloride–ether (2:1); D = chloroform–benzene–ether (1:1:1).

O-Demethylrotenone was synthesized by reacting rotenone (1 g) with equimolar boron tribromide in dichloromethane (12 ml) at −10°C for 2 min, using a procedure based on that used by Taub *et al.* (1968) for comparable reactions with compounds other than rotenoids. Preparative tlc [first with hexane–ethyl acetate mixture (2:1) and then, following product recovery, with benzene–methanol mixture (40:1)] was used several times in sequence to isolate the desired product in high purity; recrystallization from ether then yielded *O*-demethyl rotenone [mp 170–1°C; ms fragments at 380 (M^+), 203, 178, 177, 175; ir spectral peaks at 3540 (OH), 1670 (C=O), 1610 and 1505 cm^{-1} (phenyl); phenol color response obtained with ferric chloride and diazotized sulfanilic acid; methylation (diazomethane in ether) produced rotenone (tlc criterion)].

6′,7′-Epoxyrotenone was prepared by reacting rotenone (0.39 g) with equimolar 3-chloroperoxybenzoic acid in dichloromethane (15 ml) at 25°C for 24 hr. The reaction mixture was poured onto a column of dry silicic acid, the column was developed with benzene–methanol mixture (20:1), and the solvent was removed from the desired eluate fractions (tlc monitoring). The acetone-soluble portion of the residue was subjected to preparative tlc [two developments with benzene–methanol mixture (40:1) on the first plate and, following product recovery, two developments with carbon tetrachloride–ether mixture (2:1) on the second plate] and recrystallization from methanol to obtain 6′,7′-epoxyrotenone [needles, mp 176–7°C; *ca.* 5% yield; $[\alpha]^{25}D$ −133°C in chloroform; ms fragments at 410 (M^+), 219, 192; ir very similar to rotenone with peaks at 1670 (C=O), 1605 and 1505 (phenyl), 2850 and 1250 cm^{-1} (aryl-OCH_3); nuclear magnetic resonance spectrum appropriate for the assigned structure]. The α and β isomers of 6′,7′-epoxyrotenone were separated by tlc using six sequential developments with carbon tetrachloride–ether mixture (3:1). [The characterization of the two stereoisomers, which appear in about a 1:1 ratio, is given by Unai *et al.* (1972).]

Thin-Layer Chromatography. Tlc utilized 20 × 20 cm silica gel F_{254} chromatoplates (Brinkmann Instruments Inc., Westbury, N.Y.) having 0.25-mm gel thickness for analysis or 0.5- or 2-mm gel thickness for preparative separations. The solvent systems used and the R_f values for authentic neutral compounds were those listed in the footnote to Table I. Difficulties in resolving compounds with similar R_f values in each solvent system were partially overcome by multiple, sequential developments using various combinations of the individual solvent systems. The acidic compounds, separated by using benzene (saturated with formic acid)–ether mixture (10:3), gave R_f values as follows: derrisic acid, 0.13; 4,5-dimethoxysalicylic acid, 0.32; rissic acid, <0.01; toxicaric acid, 0.22; tubaic acid, 0.54. Each of the neutral products listed in Table I and the acidic products quench gel fluorescence when viewed under short wavelength uv light and, with the exception of rotenonone, they give yellow spots on exposure to iodine vapor. A spray of 10% (w/v) phosphomolybdic acid in ethanol followed by heating for 10 min at 110°C produces reddish spots with all compounds listed in Table I and with the acidic compounds indicated above with the exceptions of *O*-demethyl rotenone and derrisic acid, which give blue spots and rotenonone, which yields little color. A fresh solution of ferric chloride in methanol (1%, w/v) gives a reddish violet spot with *O*-demethylrotenone, reddish brown with derrisic acid, blue with 4,5-dimethoxysalicylic acid, and violet with tubaic acid. Diazotized sulfanilic acid (1%, w/v, aqueous) is useful for detecting phenolic products.

Spectral Analysis. Infrared spectra were determined on 5–10% (w/v) chloroform solutions of the compounds with the Perkin-Elmer Model 457 grating spectrometer. Ultraviolet spectra were determined with the Bausch & Lomb Spectronic 505 spectrophotometer using ethanol solutions of the compounds. Mass spectra were determined with the 21-103-C Mass Spectrometer (Consolidated Electrodynamic, Monrovia, Calif.). Optical rotations were measured with the ETL-NPL Automatic Polarimeter Type 143A (Bendix Ericsson, U.K. Ltd., Nottingham, England).

Photodecomposition of Rotenone in Methanol or Benzene Solution Irradiated with Ultraviolet Light. Rotenone (1 g) in methanol (600 ml) in a water-cooled, double-walled, clear, fused-quartz immersion well was irradiated for 2 hr with a high pressure, mercury vapor lamp (450 W, Corex filter to exclude light below 260 nm) while oxygen was bubbled through the solution. [In the photolysis period, the color of the solution changed from colorless to yellow and, finally, to reddish brown, and there was almost complete destruction of rotenone (tlc analysis).] Two general procedures were used to isolate photodecomposition products from the residue after evaporation of the methanol. In the first procedure, the residue was passed through a silicic acid column (2.5 × 35 cm), using 1.5 l. of benzene–methanol mixture (9:1) for elution and the products partially separated by the column (tlc analysis) were isolated by preparative tlc. In the second procedure (designed to recover carboxylic acid derivatives), the residue was dissolved in chloroform, the resulting solution was extracted with 5% aqueous potassium bicarbonate solution, and the bicarbonate solution was acidified to yield an insoluble, dark brown, pasty material which was methylated (diazomethane in ether) prior to isolation of the components by preparative tlc.

In another study, the degree of decarboxylation occurring during rotenone photodecomposition was quantitatively determined by measuring the number of moles of carbon dioxide produced per mole of rotenone. Rotenone (5 g) in methanol or benzene (600 ml) was irradiated for 2 hr with the mercury vapor lamp, with oxygenation, the carbon dioxide

produced was absorbed in barium hydroxide solution, and the dried barium carbonate was weighed; correction was made for the small amount of carbon dioxide released on irradiation of the solvent alone. Alternatively, rotenone-6*a*-^{14}C was irradiated in very dilute methanol or benzene solution for 2 hr, the $^{14}CO_2$ produced was absorbed in a methyl cellosolve–ethanolamine mixture (2:1) and the radioactivity of the mixture was measured with a liquid scintillation spectrometer.

Photodecomposition of Rotenone-6*a*-^{14}C Deposits on Silica Gel Chromatoplates, Glass Surfaces, and Bean Leaves Exposed to Various Light Sources. Rotenone-6*a*-^{14}C (10 μg) mixed with unlabeled rotenone (20 μg) and xanthone (a photosensitizer, 20 μg) was spotted on a tlc plate, exposed to sunlight for 20 hr, and the chromatoplate was developed two times in one direction with benzene–methanol mixture (40:1) and, subsequently, two times in the second direction with carbon tetrachloride–ether mixture (4:1). Radioautography and cochromatography were used for product detection and tentative identification.

The upper surface of each primary leaf (*ca.* 20 cm^2) of pinto bean seedlings was treated with rotenone-6*a*-^{14}C in ethanol to produce a uniform residual deposit of 0.5 μg per cm^2. Immediately after treatment, the plants were exposed to direct sunlight for 4 hr, the leaves were removed and rinsed in ether (5 ml × 3), and the ether was evaporated under nitrogen and the radioactive components were analyzed by tlc cochromatography with authentic unlabeled compounds, using the solvent systems given in Figure 2.

Rotenone-6*a*-^{14}C was applied in ethanol solution to glass surfaces, producing a relatively uniform deposit of 1 μg per cm^2. These rotenone deposits were exposed to three different light sources: sun, a sunlamp, and a uv lamp. Irradiation with the 275-W sunlamp (General Electric Co., Cleveland, Ohio) at a distance of 10 cm from the lamp elevated the temperature at the glass surface to approximately 75°C. Samples exposed to the uv lamp (254 nm, General Electric germicidal lamp, 15 W) were approximately 5 cm from the light source. After various exposure intervals, the reaction products were dissolved in acetone and analyzed by tlc directly, using the solvent systems shown in Figure 2. The products were tentatively identified by cochromatography.

The quantitative yields of labeled photodecomposition products relative to the amount of rotenone-6*a*-^{14}C used were determined by scraping the radioactive gel regions, detected by radioautography, from the chromatoplates into vials for radioactive counting in a liquid scintillation spectrometer.

Photodecomposition of Rotenolones in Ethanol. Each rotenolone isomer (5 mg) in ethanol (5.5 ml) in a test tube provided for the Spectronic 20 Colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.) was irradiated with short wavelength uv light (254 nm) at a distance of 8 cm. The formation of photodecomposition products was monitored by the development of color at 430 nm (a rough measure of the degree of photodecomposition). The mixture of photodecomposition products, formed from each rotenolone isomer, was also monitored, after evaporation of the ethanol, by tlc and by optical rotation in chloroform solution.

Toxicity of Photodegradation Products. The product, in dimethyl sulfoxide solution, was administered intraperitoneally to adult male white mice (Horton Laboratories, Inc., Oakland, Calif.) to determine the 24-hr LD_{50} values.

RESULTS

Photodecomposition Products from Irradiation of Rotenone in Methanol and Benzene Solutions with Ultraviolet Light.

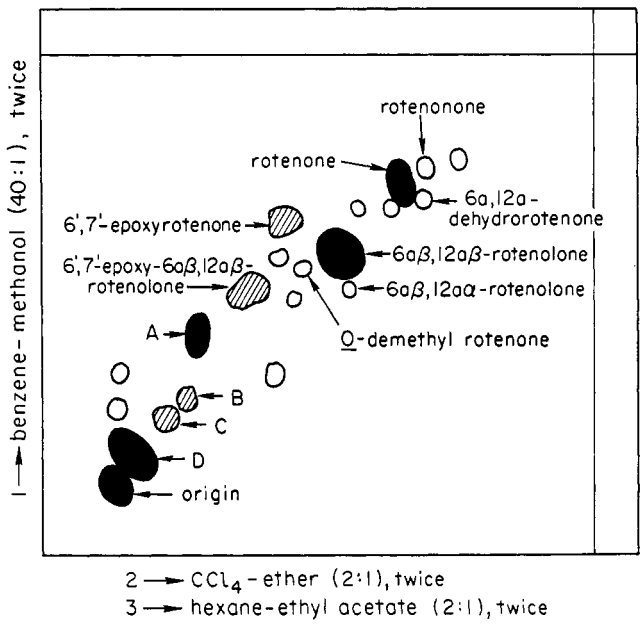

Figure 2. Diagrammatic illustration of tlc radioautogram of photodecomposition products of rotenone-6*a*-^{14}C formed on glass surfaces or bean leaves exposed to light. Amounts of products present after 4-hr exposure to sunlight on bean leaves are indicated, relatively, as percentages of the applied radiocarbon as follows: dark spots, >5%; shaded spots, 1–5%; open spots, <1%

Six photodecomposition products were isolated by column chromatography and tlc following uv irradiation of rotenone in methanol solution. Those eluted from the silicic acid column with benzene–methanol mixture, in the order of their elution, were: rotenonone; unreacted rotenone; 6aβ,12aβ-rotenolone; *O*-demethylrotenone; and tubaic acid. The acidic photodecomposition products isolated by tlc, after conversion to their methyl esters, were: rissic acid dimethyl ester; 4,5-dimethoxysalicylic acid methyl ester; and tubaic acid methyl ester. Their identity was established, in each case, by comparison with authentic compounds using various criteria, as follows: 6aβ,12aβ-rotenolone (tlc cochromatography in three solvent systems; identical ir, ms, and optical rotation); rotenonone (tlc cochromatography in three solvent systems; identical mp and ms); *O*-demethylrotenone [tlc cochromatography in three solvent systems; identical mp, ir, and ms; methylation (diazomethane in ether) produced rotenone (tlc criterion)]; tubaic acid and its methyl ester (in each case tlc cochromatography in three solvent systems; identical mp, ir, and ms); rissic acid as its dimethyl ester (tlc cochromatography in three solvent systems; identical mp, ms, and uv spectrum); 4,5-dimethoxysalicylic acid (tlc cochromatography in three solvent systems) and as its methyl ester (tlc cochromatography in three solvent systems; identical mp, ms, and uv spectrum). The following compounds were not detected by tlc (using the solvent systems given in Table I and certain other solvent systems) among the photodecomposition products, either before or after column chromatography or in either the neutral or acidic products: *trans*-rotenolones; (±)- or (−)-rotenol; (+)-6,6a-, (−)-6,6a-, or 6a,12a-dehydrorotenol; derritol; 6a,12a-dehydrorotenone; derric acid; derrisic acid; toxicaric acid.

When irradiated in benzene solution, rotenone forms a precipitate which, when recovered by dissolving in chloroform followed by evaporation of the solvent and methylation (diazomethane in ether), yields the dimethyl ester of rissic acid.

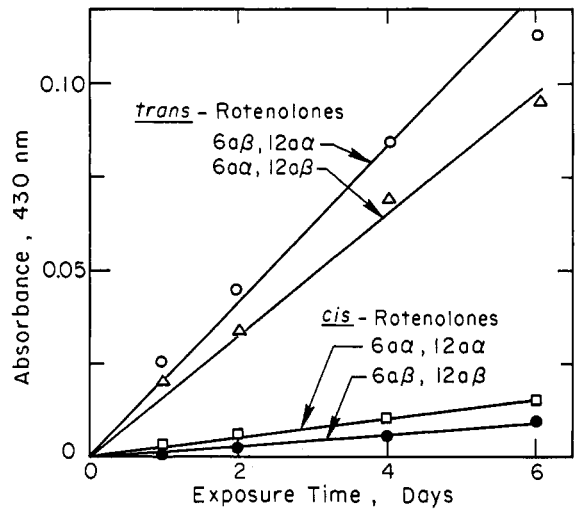

Figure 3. Rate of photodecomposition of four rotenolone isomers exposed in ethanol solution to ultraviolet light

Table II. Photodecomposition Products Formed When Rotenone-6α-^{14}C is Exposed to Light as Deposits on Glass Surfaces or on Bean Leaves

	Recovery of applied radiocarbon, %			
	Bean leaf[a]	Glass[b]		
Products	4 Hr	1 Hr	2 Hr	4 Hr
Rotenone	13.5	9.2	4.6	1.7
6a,12a-Dehydrorotenone	<0.2	<0.2	<0.2	<0.2
O-Demethyl rotenone		0.4	0.3	0.2
6′,7′-Epoxyrotenone	3.5	1.1	0.8	0.5
6′,7′-Epoxy-6aβ,12aβ-rotenolone	4.8	2.7	2.7	1.9
6aβ,12aβ-Rotenolone	11.0	13.2	5.3	2.4
6aβ,12aα-Rotenolone	0.4	0.6	0.2	0.1
Rotenonone	0.7	1.0	0.4	0.3
Unknowns				
A	7.2	3.9	4.6	4.4
B	3.7	1.2	0.9	0.6
C	3.6	2.0	1.6	1.4
D	5.9	5.1	3.6	2.6
Eight minor compounds, total	0.3	2.7	1.4	0.4
Compounds located at tlc origin	20.4	32.2	32.1	31.1
Loss (volatilization)	25.0	24.7	41.5	52.4[c]

[a] Exposed to sunlight only. [b] Exposed to sun, sunlamp and uv (254 nm) irradiation. The average result is presented because the extent of degradation and the ratio of the products formed are similar with the different light sources. [c] The loss on 4-hr exposure in the dark was 5–10%.

Extraction of the irradiated benzene solution with 5% potassium bicarbonate and esterification of the recovered acids with diazomethane yields the methyl esters of tubaic and 4,5-dimethoxysalicylic acids. Carbon dioxide is also found as a rotenone photodecomposition product, amounting to 12 and 5%, respectively, on a mole-for-mole basis when the unlabeled rotenone is irradiated for 2 hr in methanol and benzene solutions. A portion of the carbon dioxide originates from the 6a position because labeled rotenone, irradiated in very dilute methanol and benzene solutions, yields 2.5 and 1.1% $^{14}CO_2$, respectively, within 2 hr.

The failure to isolate *trans*-rotenolones suggests that they may be unstable on exposure to uv light, a proposal confirmed by separately irradiating each of the four rotenolone isomers in ethanol solution with uv light. During irradiation, solutions of the trans but not the cis isomers change from colorless to yellow. [The yellow color measured at 430 nm (Figure 3) might result from dehydration of the rotenolones to 6a,12a-dehydrorotenone and further oxidation to form rotenonone, both of which give yellow solutions in an organic solvent.] The optical rotation, as $[\alpha]^{25}D$ in chloroform, of the rotenolones changes on irradiation as follows: 6aβ,12aα isomer, from +420° to −48°; 6aα,12aβ isomer, from −410° to −120°; 6aβ,12aβ and 6aα,12aα isomers show little, if any, change. These results indicate extensive epimerization of the *trans*- but not of the *cis*-rotenolones. Tlc analyses reveal about 60% conversion of 6aβ,12aα- to 6aβ,12aβ-rotenolone, about 30% conversion of 6aα,12aβ- to 6aα,12aα-rotenolone, but no significant photodecomposition products are obtained from the *cis*-rotenolones. Four other photoproducts form on irradiation of 6aβ,12aα- and 6aα,12aβ-rotenolones; these are small amounts of 6a,12a-dehydrorotenone and rotenonone plus two major products of lower R_f values than the parent compound. Although not identified, it is known (tlc) that the two unknowns are not derritol, derrisic acid, or the 6aα,5′β- or 6aβ,5′β-ketoaldehyde derivatives of rotenone. Thus, each of several types of analyses indicates the photoinstability of *trans*-rotenolones; so, the *trans*-rotenolones formed on rotenone photodecomposition are expected to be transient intermediates resulting in accumulation of only the cis isomers, particularly 6aβ,12aβ-rotenolone.

Photodecomposition Products from Exposure of Rotenone Deposits on Silica Gel Chromatoplates, Glass Surfaces, and Bean Leaves to Various Light Sources. The exposure of rotenone-6α-^{14}C mixed with xanthone to sunlight on silica gel chromatoplates yields at least ten products, including ones cochromatographing with 6aβ,12aβ-rotenolone, 6aβ,12aα-rotenolone, rotenonone, and 6′,7′- epoxyrotenone.

The same photodegradation products are formed during the exposure of rotenone deposits on bean leaf surfaces to sunlight or on glass surfaces to sunlight, light from the sunlamp, or uv irradiation (Table II, Figure 2). The ratio of products depends more on the amount of residual rotenone than on the nature of the surface or the type of light used. The major products identified by cochromatography are, in each case, 6aβ,12aβ-rotenolone, 6′,7′-epoxyrotenone, and 6′,7′-epoxy-6aβ,12aβ-rotenolone (each epoxide possibly a mixture of α and β isomers), and rotenonone; 6aβ,12aα-rotenolone, *O*-demethylrotenone, and 6a,12a-dehydrorotenone are minor products. There are also many other minor photodegradation products (chromatographic positions shown by circles not designated by letters in Figure 2). The possible presence or absence of certain other compounds among the photodecomposition products is established with varying degrees of certainty; 8′-hydroxyrotenone is not detected as a photoproduct; 8′-hydroxy-6aβ,12aβ-rotenolone is possibly present in trace amounts; 6′,7′-dihydro-6′,7′-dihydroxyrotenone and its 6aβ,12aβ-rotenolone derivative are minor products or are absent (they appear in a low R_f chromatographic region containing many labeled products). There are also other degradation products not detected because they lack the labeled 6a position.

Toxicity of Photodegradation Products. Preliminary studies show that, on irradiating rotenone in methanol solution with uv light, the acute intraperitoneal toxicity of the reaction mixture (after evaporation of the solvent) to mice decreases with increasing irradiation time. Thus, the mixture of photodegradation products is less toxic than rotenone. The LD_{50} values are 7.2 and 8.0 mg per kg for 6′,7′-epoxy-

rotenone and *O*-demethylrotenone, respectively, *vs.* 2.8 for rotenone. From another study, it is known that the comparable value for rotenolone I (6aβ,12aβ-rotenolone combined with some 6aα,12aα-rotenolone) is 4.1 mg per kg (Fukami *et al.*, 1967).

DISCUSSION

Several photochemical reactions occur when solutions or residual deposits of rotenone are irradiated in the presence of oxygen (Figure 1). These reactions include: *O*-demethylation, probably at the 3 position; epoxidation at the 6′,7′ position; hydroxylation at the 12a position, producing epimeric rotenolones; dehydration of the rotenolone(s); oxidation of 6a,12a-dehydrorotenone at the 6 position, producing rotenononе; ring cleavage at the 6a,12a position of 6a,12a-dehydrorotenone or rotenononе to form benzoic acid derivatives; decarboxylation of certain photodegradation products at the 6a position. Also, there are many uncharacterized products which possibly are formed by various combinations of attack at the sites already established. The photoreaction products are oxidized derivatives; so, oxygen plays a critical role.

The principal photochemical reactions of rotenone are probably initiated by abstraction of the hydrogen at position 12a by another rotenone molecule in an excited electronic state. [Rotenone acts as a photosensitizer in other photochemical reactions (Ivie and Casida, 1970, 1971).] The reaction of molecular oxygen with the newly created radical intermediate (Ia, Figure 4) then has the possibility of forming two epimeric hydroperoxide intermediates which can decompose to the corresponding epimeric rotenolones (Figure 4). [While it is conceivable that radical intermediate Ia might undergo ring opening (Ib, Figure 4) and recyclization to another radical intermediate (Ic, epirotenone with the 12a-hydrogen abstracted, Figure 4), this does not occur to an appreciable degree under the conditions studied because the 6aα,12aβ- and 6aα,12aα-rotenolone isomers are not produced in detectable yields.] Dehydration of 6aβ,12aα-rotenolone, *via* trans elimination, is established as one source of 6a,12a-dehydrorotenone. The production of only small amounts of 6a,12a-dehydrorotenone is probably the result of its transient nature as an intermediate, an allylic hydrogen being abstracted from the 6 position, and the radical produced undergoing attack by oxygen to form the 6-hydroperoxide and, ultimately, rotenononе. (As described above, 6aβ,12aα-rotenolone photodecomposes to rotenononе.) 6a,12a-Dehydrorotenone might also undergo a photosensitized oxygen transfer reaction *via* the 6a,12a-epoxide leading to ring cleavage and formation of rissic and tubaic acids. Alternatively, the 12a-hydroperoxide possibly is the intermediate for the 6a,12a-epoxide or undergoes direct ring cleavage leading to the indicated acids. Rotenononе might also form a 6a,12a-epoxide that oxidizes, ultimately, to tubaic acid, 4,5-dimethoxysalicylic acid, and oxalic acid, the latter being subsequently oxidized to carbon dioxide. On the other hand, the rissic acid may photodecompose directly to give 4,5-dimethoxysalicylic and oxalic acids, based on analogy with other phenoxyacetic acids (Kelly and Pinhey, 1964). Rotenone also undergoes photochemical demethylation and this probably involves the same short-lived radical intermediate (Ia, Figure 4) formed on abstraction of the 12a-hydrogen from rotenone. Accordingly, the delocalization of the unpaired electron *via* π orbitals would facilitate cleavage of the methoxyl group in the position para to the benzylic radical (*i.e.*, at the 3 position), producing the indicated isomer for *O*-demethylrotenone. While 6′,7′-epoxy-

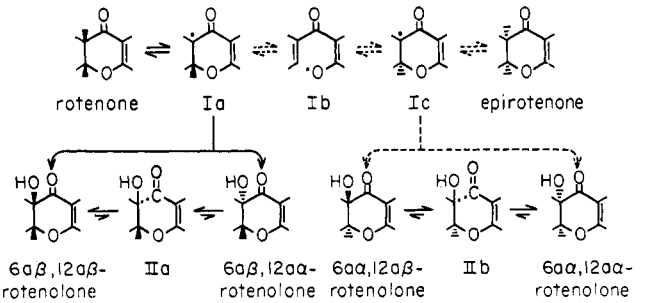

Figure 4. Scheme for photochemical formation of rotenolones and their photochemical epimerization

6aβ,12aβ-rotenolone can be formed from either 6aβ,12aβ-rotenolone or 6′,7′-epoxyrotenone, it appears likely that most of the former compound arises from the rotenolone.

The *trans*- but not the *cis*-rotenolone isomers decompose readily on irradiation in ethanol solution with uv light. Also, there is photoepimerization of the *trans*-rotenolone diastereoisomers to the cis isomers upon exposure to uv light. As shown in Figure 4, the photoepimerization probably involves fission of the 12,12a bond, which has a low bond-dissociation energy because of the adjacent carbonyl and benzylic alcohol groups. Formation of the more stable *cis*-rotenolones from the diradical intermediates (IIa or IIb, Figure 4) is favored. The trans isomers, but not the cis isomers, show a prominent ms peak at *m/e* 392 (*i.e.*, dehydration to give 6a,12a-dehydrorotenone), which is consistent with the greater stability of the *cis*-rotenolones compared to the *trans*-rotenolones. The greater stability of cis isomers than trans isomers is also revealed by oxygenation of rotenone in the presence of a catalytic amount of alkali in ethanol solution, which produces approximately 20% of *trans*- and 76% of *cis*-rotenolones. Natural rotenone has the thermodynamically stable cis configuration. [The unstable trans configuration has not been isolated from natural sources (Crombie, 1963).]

Rotenone-treated crops are expected to contain a great variety of rotenone-derived photodecomposition products. The available information on the toxicology of these products (Fukami *et al.*, 1959, 1967, 1969; Shepard and Campbell, 1932; Yamamoto *et al.*, 1971; this report) indicates that only one of them, 6aβ,12aβ-rotenolone, has high biological activity. Therefore, it is advisable to analyze for this rotenolone, as well as rotenone, in determining rotenone-derived residues on crops.

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Photochemistry of Bioactive Compounds. Photochemical Reactions of Heptachlor: Kinetics and Mechanisms

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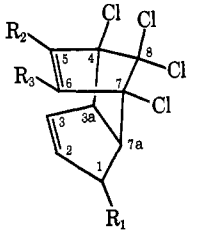
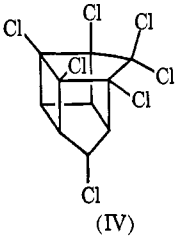
The product formation, reaction kinetics, and mechanisms of the photolysis of heptachlor (1,4,5,-6,7,8,8 - heptachloro - 3a,4,7,7a - tetrahydro - 4,7-methanoindene, I) have been investigated. Photolysis at 253.7 nm in hydrocarbon solvents yields two olefinic monodechlorination isomers (II, III) ($\Phi = 0.025$); at 300 nm in acetone, a 2 + 2 cycloaddition or cage compound (IV) is the exclusive product ($\Phi = 9.35 \times 10^{-5}$ based on total absorption of energy by acetone); and in mixed cyclohexane/acetone solutions at 300 nm, IV and a C-1 cyclohexyl adduct (V) are formed *via* a triplet and allylic free radical, respectively. A cage opening reaction of IV occurs at 200 nm to yield I ($\Phi = 0.195$). Photodechlorination to yield II and III occurs *via* excitation of the 5,6 double bond, most probably through a singlet state; while formation of IV and V occurs *via* a triplet mechanism involving the 2,3 double bond. A kinetic mechanism and specific rate constants are reported.

The products formed by the photolysis of heptachlor (I) (1,4,5,6,7,8,8 - heptachloro - 3a,4,7,7a - tetrahydro - 4,7-methanoindene) depend upon the reaction conditions. Irradiation of heptachlor at wavelengths below 260 nm in a nontriplet sensitizing solvent yields only a mixture of the two monodechlorination isomers 1,4,5,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (II) and 1,4,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (III) in equal amounts.



I: R_1 = Cl, R_2 = Cl, R_3 = Cl
II: R_1 = Cl, R_2 = Cl, R_3 = H
III: R_1 = Cl, R_2 = H, R_3 = Cl
V: R_1 = S, R_2 = Cl, R_3 = Cl

If the reaction is carried out at higher wavelengths (300 nm) in a triplet-sensitizing solvent such as acetone, the only product is a cage compound, 1,2,3,6,9,10,10-heptachloropentacyclo(5.3.0.$0^{2,5}$.$0^{3,9}$.$0^{4,8}$) decane (IV). Although this cage compound is also formed upon irradiation (300 nm) of heptachlor solutions in mixtures of acetone and cyclohexane, the principal product formed under these conditions is a substitution product (V) where the allylic chlorine of carbon-1 is replaced by a cyclohexyl (-S) group (McGuire *et al.*, 1970).



(IV)

Anderson *et al.* (1968) have investigated the dependence of photoproduct formation on the reaction conditions for systems analogous to heptachlor and have proposed a singlet transition state for the photodechlorination and a triplet state for cage formation. A study of the kinetics of these heptachlor photolyses should further delineate the mechanisms involved.

EXPERIMENTAL

Materials. A commercial sample of heptachlor, 25% by weight, was dissolved in acetone, filtered, and the solvent evaporated. The resulting solid was redissolved in *n*-hexane

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