Syntheses and Resolution of Optically Active DDT Analogs and Their Toxicity to the Housefly, *Musca domestica* L.

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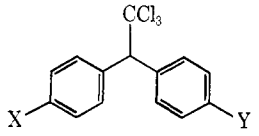
The syntheses of several optically active DDT analogs have been accomplished and their resolved enantiomers have been tested for toxicity in the housefly, *Musca domestica* L. Substitution of a hydrogen for a halogen on one of the phenyl rings resulted in a reduction of toxicity. The *d*- and *l*-chlorobromo derivative were equitoxic to authentic DDT (0.39–0.45 μg/fly) while the *d*- and *l*-fluorobromo analogs were slightly less toxic (0.66–0.70 μg/fly).

It has long been known that not only DDT but also many of its derivatives, including unsymmetrical analogs such as I, are insecticidally active. It has also been shown that DDT and some of its derivatives are enzymatically de-



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hydrochlorinated to their nontoxic, ethylenic analogs by houseflies and other insects (Lipke and Kearns, 1959) and by oxidative detoxication by the body louse (Perry *et al.*, 1963). Investigation of the unsymmetrical derivatives in the past has been limited to the racemic compounds (Metcalf, 1955). More recent work by Metcalf *et al.* (1971) has shown that microsomal multifunction oxidases hydroxylate 1-*p*-chlorophenyl-1-phenyl-2,2,2-trichloroethane effectively in the unsubstituted *p* position. Too, its toxicity was synergized 40 times by piperonyl butoxide. It is of interest to determine whether the individual enantiomers of an enantiomeric pair show any differences in either their ease of dehydrochlorination or their toxicity. This paper reports the syntheses of four enantiomeric pairs of DDT derivatives and their toxicity to adult houseflies, *Musca domestica* L.

MATERIALS AND METHODS

The syntheses of these compounds are outlined in Figure 1. The carbinol(II) was prepared in 93% yield by the condensation of *p*-tolualdehyde with chloroform in the presence of potassium *tert*-butoxide (Reeves and Fine, 1964).

The condensation of the carbinol(II) with the appropriate aromatic hydrocarbons gave III and IV in 63 and 71% yields, respectively.

Several methods of oxidation of the hydrocarbons to the corresponding carboxylic acids were investigated. The direct oxidation of the methyl group with either acidic potassium permanganate or potassium dichromate under a variety of conditions was found to be of no preparative value. A two-stage oxidation which involved the intermediate aldehyde gave, in each case, carboxylic acid in fair yield. The hydrocarbons (III and IV) were first oxidized with chromium trioxide to the aldehydes (V and VI) (Perron and Barre, 1952) and then to the acids [VII (48%) and VIII (33%)] with acidic potassium permanganate.

The acids were converted to the amines [IX (93%) and X (82%)] *via* the Schmidt reaction using trifluoroacetic acid (Moritsugu, 1954) as the solvent. The amines were converted to their tartrate salts and the diastereomeric pairs were separated by recrystallization from ethanol. Neutralization of each of the four optically resolved tartrate salts gave an optically active amine (*d*-IX, *l*-X, and *l*-IX, *d*-X). The properties of the amines and their corresponding tartrate salts are shown in Table I.

Each optically resolved amine was diazotized and then deaminated in the presence of hypophosphorus acid. The DDT derivatives (*d*-XI, *l*-XI, *d*-XII, and *l*-XII) which resulted from these reactions are listed in Table II.

When the diazonium bromides were treated with cuprous bromide and heated, the corresponding bromo derivatives (*d*-XIII, *l*-XIII, *d*-XIV, and *l*-XIV) were formed. The results of these experiments are shown in Table II.

Mass spectra were obtained using an LKB-9000 and a Dupont 21-490 mass spectrometer. Samples were introduced *via* a direct probe into the ionization source. Spectra were run at an ionization voltage of 70 electron volts (eV).

The houseflies, *Musca domestica* L., used were an insecticide-susceptible strain routinely reared on CSMA medium (Peet-Grady, 1959) and the adults were fed a 1:1 mixture of sucrose and nonfat dry milk. The rearing and testing rooms were maintained at 27 ± 2° and *ca.* 50% relative humidity. Only 3- to 4-day-old female flies were used.

Twenty-five randomly selected flies, anesthetized by carbon dioxide, were treated on the mesonotum with the test compound in 1 μl of acetone with an automatic calibrated microsyringe (Biotronics Microapplicator, Brookings, S.D.). The flies were then placed into screened pint containers provided with water, and moribund and/or dead flies were recorded 24 hr after treatment. The LD_{50}'s of the compounds were determined by plotting the mean percent mortalities converted to probits *vs.* log dosage.

All compounds were applied as an acetone solution, and flies treated with acetone only served as controls. All tests were conducted in triplicate.

EXPERIMENTAL SECTION

All melting points are uncorrected. All microanalyses were by Galbraith Laboratories, Inc., Knoxville, Tenn. Ir spectra were obtained with a Perkin-Elmer 337 instrument. All specific optical rotations were obtained with a Bendix Automatic Polarimeter, model 143A. All rotations were taken in 95% ethanol. The term "worked up in the usual

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Table I. Properties of the Optically Active Amines and Their Tartrate Salts

Compound	mp, °C	Optical rotation: α at 546.1 nm	Optical rotation: Temp, °C
d-Tartrate salt	144–146	−17.4	23
l-IX	149.1–149.5	−31.3	22
l-Tartrate salt	147–148	+20.1	27
d-IX	152.5–152.6	+34.8	23
d-Tartrate salt	143–144	−9.6	24
l-X	96.3–97	−17.4	27
l-Tartrate salt	141–143	+9.6	24
d-X	96–97	+17.4	24

manner" means that a solution of the products in an organic solvent after washing with dilute alkali and/or acid, water, and saturated salt solution was filtered through anhydrous magnesium sulfate and the solvent was removed by distillation.

2,2,2-Trichloro-1-(*p*-tolyl)ethanol (II). To a stirred solution of 400 g (3.3 mol) of *p*-tolualdehyde was added at 0–6° over a 5-hr period a solution of potassium *tert*-butoxide prepared from 233 g (5.9 mol) of potassium and 5 l. of *tert*-butyl alcohol. Benzene, 1200 ml, was then added and the reaction mixture was stirred for 2.5 hr at 0°. At the end of this time the reaction mixture was poured into ice water containing 159 ml of concentrated sulfuric acid. The organic layer was extracted with ether and worked up in the usual manner. Distillation gave 744 g (93%) of product II, bp 106–116° (2 mm).

Treatment of II with acetic anhydride followed by recrystallization from ethanol gave the colorless acetate, mp 109.5–110.5°.

Anal. Calcd for $C_{11}H_{11}O_2Cl_3$: C, 46.9; H, 3.9; Cl, 37.8. Found: C, 46.8; H, 3.8; Cl, 37.8.

The 2,2,2-trichloro-1-(*p*-tolyl)ethanol and its acetate have been previously characterized by Bergmann *et al.* (1950).

1,1,1-Trichloro-2-(*p*-chlorophenyl)-2-(*p*-tolyl)ethane (III). To a well-stirred mixture of 300 g (1.3 mol) of II and 750 ml of chlorobenzene at 0° was added dropwise over 2 hr 1200 ml of concentrated sulfuric acid. The reaction mixture was stirred for 22 hr at 0° and then poured into ice water. The organic layer which separated was extracted into ether and worked up in the usual manner. The residual oil was crystallized from ethanol to give 267 g (63%) of III as colorless crystals, mp 98–99°.

Anal. Calcd for $C_{15}H_{12}Cl_4$: C, 53.9; H, 3.6. Found: C, 53.4; H, 3.5.

1,1,1-Trichloro-2-(*p*-fluorophenyl)-2-(*p*-tolyl)ethane (IV). Condensation of the carbinol(II) with fluorobenzene by the procedure described above gave 71% of IV as colorless crystals, mp 52–54°. Recrystallization from ethanol gave an analytical sample, mp 59.1–59.5°.

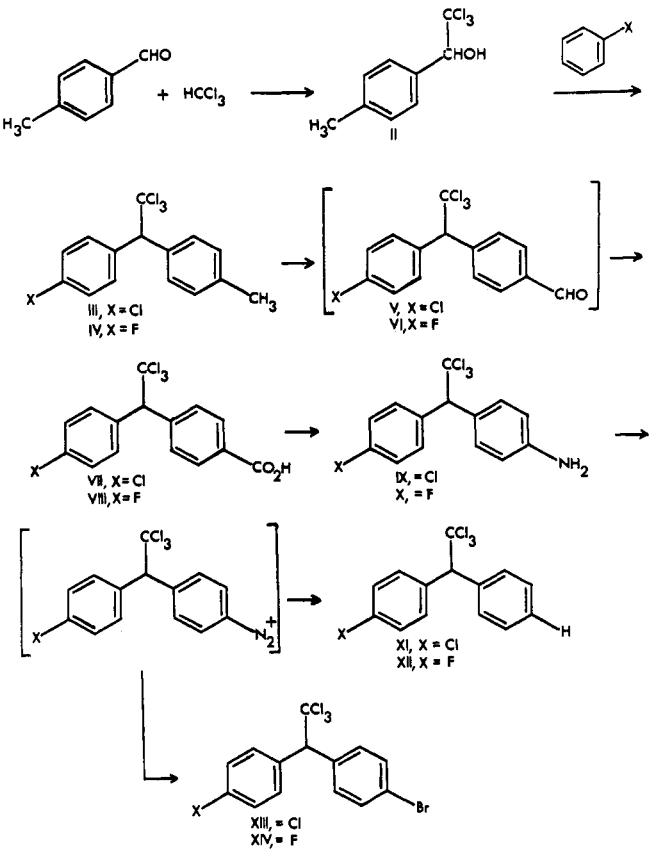

Figure 1. Preparative sequence for the synthesis of the optically active isomers of DDT

Anal. Calcd for $C_{15}H_{12}Cl_3F$: C, 56.7; H, 3.8; Cl, 33.4. Found: C, 56.9; H, 3.7; Cl, 33.4.

1,1,1-Trichloro-2-(*p*-carboxyphenyl)-2-(*p*-chlorophenyl)ethane (VII). To a cooled (0–5°) well-stirred mixture of 11.1 g (0.03 mol) of III, 75 ml of acetic anhydride and 25 ml of glacial acetic acid, was added 10 ml of concentrated sulfuric acid followed by 12 g of chromium trioxide. When the addition was complete, stirring was continued for 15 min at 0° and the product was then poured over crushed ice. The crude diacetate, an oil, was separated and dissolved in a mixture of 375 ml of ethanol, 240 ml of water, and 23 ml of concentrated sulfuric acid. After a 1.5-hr reflux period, the mixture was cooled to give 11.0 g of a tan solid V, mp 75–81°. The 2,4-dinitrophenylhydrazone of V, after several recrystallizations from ethanol, melted at 239–240°.

Table II. Experimental Data of the DDT Homologs

Derivative	Compound	X	Product	Yield, %	mp, °C	Optical rotation: α at 546.1 nm	Optical rotation: Temp, °C
Hydrogen	*l*-IX	Cl	*l*-XI[a,b]	49	60.5–61.5	−4.2	25
	d-IX	Cl	*d*-XI[a,b]	33	66.5–67.0	+4.1	24
	l-X	F	*l*-XII[a,b]	88	35–36.5	−12.1	26
	d-X	F	*d*-XII[a,b]	84	36–38	+12.5	26
Bromo	*l*-IX	Cl	*l*-XIII[c,d]	51	123–124	−1.0	26
	d-IX	Cl	*d*-XIII[c,d]	40	123–124	+3.5	26
	l-X	F	*l*-XIV[c,d]	56	64–65	−22.8	24
	d-X	F	*d*-XIV[c,d]	53	61–64	+22.8	24

[a,c] The ir spectra of these compounds were identical to those of the corresponding racemates. The racemates were prepared by modification of the method of Chattaway and Muir (1934). [b] Racemates: XI, mp 74° (Chattaway and Muir, 1934); XII, mp 50–52° (Balaban and Sutcliffe, 1948). [d] Racemates: XIII, mp 124.3–124.5° (Schneller and Smith, 1948); XVI, mp 63–64° (*Anal.* Calcd for $C_{14}H_9Cl_3BrF$: C, 44.0; H, 2.4; Cl, 27.8. Found: C, 43.8; H, 2.2; Cl, 27.6).

Table III. Summary of the Major Mass Spectral Fragments for DDT and the Optically Active Analogs of DDT

Compound	P		PCl		PCl_2		PCl_3		$PCCl_3$		$PCHX_4$		$PCHX_5$		Misc	
	M	%[a]	M	%	M	%	M	%	M	%	X_4/M	%	X_5/M	%	M	%
DDT	352	17	317	17	282	20	247	5	235	100	Cl_4 199	7	Cl_5 165	39		
d-XI	318	3	283	2	248	5	213	8	201	100	Cl_4 165	29				
l-XI	318	4	283	2	248	4	213	6	201	100	Cl_4 165	27				
dl-XI	318	>1	283	>1	248	3	213	9	201	100	Cl_4 165	43				
d-XII	302	>1	267	3	232	4	197	18	185	100	Cl_3F 165	26				
l-XII	302	>1	267	4	232	7	197	18	185	100	Cl_3F 165	33				
dl-XII	302	6	267	5	232	12	197	73	185	96	Cl_3F 165	100				
d-XIII	396	2	361	3	326	6	291	6	279	75[b]	Cl_4 243	8	Cl_4Br 165	58	212 175	20 25
l-XIII	396	2	361	3	326	5	291	4	279	75[b]	Cl_4 243	5	Cl_4Br 165	70	212 175	16 15
dl-XIII	396	3	361	2	326	3	291	7	279	77[b]	Cl_4 243	10	Cl_4Br 165	63	212 175	23 24
d-XIV	380	2	345	2	310	7	275	8	263	100	Cl_3Br 183	55	Cl_3BrFl 165	>1	196	23
l-XIV	380	>1	345	3	310	20	275	7	263	100	Cl_3Br 183	48	Cl_3BrFl 165	>1	196	45
dl-XIV	380	3	345	3	310	7	275	6	263	100	Cl_3Br 183	49	Cl_3BrFl 165	>1	196	20

[a] Relative abundance as percent of base peak. [b] P + 2 (M = 281) was the base peak.

Anal. Calcd for $C_{21}H_{14}O_4N_4Cl_4$: C, 47.8; H, 2.7; N, 10.6; Cl, 26.9. Found: C, 47.9; H, 2.9; N, 10.9; Cl, 27.3.

To a cooled (15°) stirred mixture of 7.29 g (0.02 mol) of V, 70 ml of water and 7 ml of concentrated sulfuric acid, was added 2.1 g of potassium permanganate. The reaction mixture was stirred for 2 hr at room temperature and extracted with ether–benzene mixture, and the manganese dioxide was separated by filtration. The organic extract was treated with 10% sodium carbonate. The aqueous wash, along with the precipitated sodium salt, was acidified and the crude solid which resulted was recrystallized from an ethanol–water mixture to give 3.5 g (48%) of the acid VII, mp 169–170. Repeated recrystallization from ethanol gave an analytical sample, mp 171.2–171.8°.

Anal. Calcd for $C_{15}H_{10}O_2Cl_4$: C, 49.5; H, 2.8; Cl, 39.0. Found: C, 49.6; H, 2.6; Cl, 39.4.

1,1,1-Trichloro-2-(*p*-carboxyphenyl)-2-(*p*-fluorophenyl)ethane (VIII). Oxidation of IV by the procedure described above gave 33% of the acid VIII, mp 160–163°. Recrystallization from ethanol gave an analytical sample, mp 161.8–162.2°.

Anal. Calcd for $C_{15}H_{10}O_2Cl_3F$: C, 51.9; H, 2.9; Cl, 30.6; F, 5.5. Found: C, 51.8; H, 2.9; Cl, 30.5; F, 5.6.

1,1,1-Trichloro-2-(*p*-aminophenyl)-2-(*p*-chlorophenyl)ethane (IX). To a stirred mixture of 76 g (0.2 mol) of VII, 380 ml of trifluoroacetic acid and 100 ml of concentrated sulfuric acid maintained at 60°, was added over 30 min 20.3 g of sodium azide. Heating was continued until the evolution of gas became negligible (6 hr). The reaction mixture was then poured over crushed ice. The organic layer which resulted was worked up in the usual manner to give 65 g (93%) of the amine IX, mp 148–150°. Recrystallization from ethanol gave an analytical sample, mp 147.4–148.2°.

Anal. Calcd for $C_{14}H_{11}NCl_4$: C, 50.2; H, 3.3; N, 4.2. Found: C, 50.2; H, 3.3; N, 4.1.

1,1,1-Trichloro-2-(*p*-aminophenyl)-2-(*p*-fluorophenyl)ethane (X). Treatment of the acid (VIII) with sodium azide as described above gave the amine X, mp 107–109° in 82% yield. Recrystallization from ethanol gave an analytical sample, mp 112.4–112.5°.

Anal. Calcd for $C_{14}H_{11}NCl_3F$: C, 52.8; H, 3.5; Cl, 33.4; N, 4.4. Found: C, 53.0; H, 3.5; Cl, 33.5; N, 4.5.

Resolution of IX and X. A mixture of equimolar amounts of the amine IX or X and *d*-tartaric acid was dissolved in boiling ethanol. Concentration of the solution gave the tartrate salt of the amine as a light-colored solid. Repeated recrystallization from ethanol gave resolved levorotatory tartrate salt. The salt was shaken for 5 min with cold 10% hydrochloric acid and the suspension which resulted was poured into 10% sodium carbonate. After vigorous agitation for 10 min the mixture was extracted with ether and worked up in the usual manner. Removal of the ether at reduced pressure gave the resolved levorotatory amine [*l*-IX (30%); *l*-X (21%)].

Crude partially resolved dextrorotatory amine was recovered from the combined tartrate mother liquors without further purification and in the presence of *l*-tartaric acid converted to its tartrate salt. When treated as described above, resolved dextrorotatory amine [*d*-IX (15%); *d*-X (31%)] was obtained. The melting points and optical rotations of the resolved tartrate salts and amines of IX and X are shown in Table I. The ir spectra of the resolved amines were identical to those of the corresponding racemates.

Deamination of the Resolved Amines. To a cooled solution (0°) of the amine (3 mmol) and 30 ml of 12 *N* sulfuric acid in 75 ml of glacial acetic acid was added 0.3 g of sodium nitrite. The solution was stirred for 40 min at 0°; 45 ml of 50% hypophosphorus acid was added and then allowed to stand at 5° for 23 hr. At the end of this time the reaction mixture was diluted with water, extracted with ether, and worked up in the usual manner to give a light-colored solid. Column chro-

matography (1 × 15 cm) of this material on 80–200 mesh Fisher acid-washed alumina (petroleum ether, 40–60°) followed by recrystallization from ethanol gave the product as a colorless solid. The products of the deamination reactions are described in Table II.

Sandmeyer Reactions of the Resolved Amines. To a cooled solution (−5°) of the amine (3 mmol) and 3 ml of 48% hydrobromic acid in 50 ml of propionic acid was added 0.3 g of sodium nitrite. The solution was stirred for 1 hr at −5° and then 0.9 g of freshly prepared cuprous bromide was added. After being stirred for an additional hour at −5°, the reaction mixture was heated to 95° for 15 min and then poured into ice water. The mixture was extracted with ether and worked up in the usual manner to give a light-colored solid. Column chromatography of this material on Fisher acid-washed alumina (petroleum ether, 40–60°) followed by recrystallization from ethanol gave the product as a colorless solid. The products of the Sandmeyer reactions are described in Table II.

RESULTS AND DISCUSSION

Table III lists the major mass spectral fragments and their relative abundance for DDT and the optically active analogs of DDT. The mass spectra of the DDT enantiomers are straightforward, showing the usual successive loss of chlorines from the CCl_3 group with the base peak as the loss of the CCl_3 group (except for *dl*-XII). All of the isotopic ratios of both the parent and major fragments were as expected. The loss of a fluorine is definitely not favored, as shown in Table III.

The toxicity of authentic DDT and the optically active analogs of DDT in the housefly are presented in Table IV. The DDT standard produced an LD_{50} of 0.47 μg/fly. Only XIII demonstrated equal toxicity against the flies (0.29–0.45 μg/fly) and there were no differences in toxicity between the enantiomeric forms. Compound XIV also demonstrated toxicity to the fly, but less so than XIII (0.66–0.70 μg/fly). Again there were no demonstrable differences between the enantiomers.

Compounds XI and XII were much less toxic than DDT. The racemates of compound XI were equitoxic (*ca.* 5 μg/fly), while compound XII was more toxic than XI, with the *d* enantiomer and *dl* mixture equitoxic (26.0 μg/fly) and the *l* enantiomer most toxic (17.0 μg/fly).

The results indicate that substitution of a hydrogen for a halogen on one of the phenyl rings decreases the toxicity of that compound to the housefly, but only compound XII gives indication of a stereospecific effect in regards to toxicity (*l*-XII was more toxic than the *d* racemate). Whether or not such differences are due to different rates of absorption through the fly cuticle, translocation differences, or differences in mode of toxic action is not known and must await future metabolic studies.

These studies demonstrate that the chlorobromo derivatives were better than the fluorobromo compounds. Too, these racemates (XIII and XIV) were of equal toxicity when tested against the housefly, indicating no differences in stereospecificity.

Table IV. The Toxicity of DDT and Optically Active Analogs of DDT to the Housefly

Compound	LD_{50}, μg/fly
DDT	0.47
d-XI	5.20
l-XI	5.10
dl-XI	5.00
d-XII	26.00
l-XII	17.00
dl-XII	26.00
d-XIII	0.39
l-XIII	0.40
dl-XIII	0.45
d-XIV	0.66
l-XIV	0.70
dl-XIV	0.66

These studies have added information in the insecticide mode of action research and future investigations involving metabolism of optically active racemates of DDT analogs could prove to be highly useful in explaining the toxic action of DDT in animals.

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