α-Trichloromethylbenzylanilines and α-Trichloromethylbenzyl Phenyl Ethers with DDT-Like Insecticidal Action

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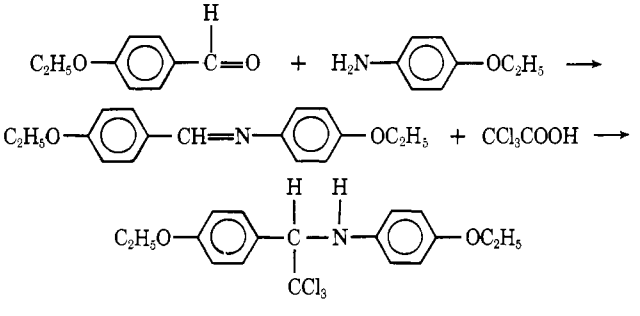
A series of *p,p'*-disubstituted α-trichloromethylbenzylanilines which closely resemble DDT-type compounds in structure have been synthesized and evaluated for insecticidal activity. These compounds affect insects in a manner indistinguishable from DDT, and the most effective compounds such as α-trichloromethyl-*p*-ethoxybenzyl-*p*-methylaniline were of comparable toxicity with DDT to flies and mosquito larvae. The metabolism of ^3H-α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline was studied in the housefly, salt-marsh caterpillar, mouse, and in a model ecosystem where the compound was substantially biodegradable. A comparable series of α-trichloromethylbenzyl phenyl ethers was also shown to have DDT-like action.

In the search for persistent yet biodegradable insecticides with DDT-like action we have considered a series of α-trichloromethylbenzylanilines. Because of the configuration of the benzylaniline moiety these compounds have a pronounced structural resemblance to DDT, as shown in Figure 1. Their insecticidal action is quantitatively very similar to DDT in that they produce the characteristic neuromuscular disturbances leading to hyperexcitability, incoordination, prostration, and death. This is associated with electrophysiological manifestations of multiple volleys of impulses from the nerve axons of poisoned houseflies which are qualitatively and quantitatively indistinguishable from those produced by DDT (Miller, 1972). The total sequence of symptoms of poisoning in the housefly, as analyzed by an "insectograph," is indistinguishable from DDT. These α-trichloromethylbenzylanilines are much less toxic to the mouse than DDT and are biodegradable because of the easy cleavage of the $-NC(CCl_3)-$ bond. The finding that DDT-like activity is not appreciably disturbed by insertion of a nitrogen atom between the two aryl rings of the molecule suggested that interesting insecticides might result from similar analogs with other bridging atoms such as oxygen and sulfur. Some of these compounds have also been investigated and have been found to also have DDT-like activity.

MATERIALS AND METHODS

The α-trichloromethylbenzylanilines discussed in this paper were highly purified chemicals whose structures were confirmed by nmr spectrometry, as shown in Table I. They were synthesized in two steps: the preparation of Schiff's bases by condensing 1 mol of substituted benzaldehyde with 1 mol of substituted aniline in boiling ethyl alcohol; and treatment of the resulting benzylideneaniline with trichloroacetic acid in toluene at reflux temperature (Lukasiewicz, 1964). The preparation of α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline is typical. *p*-Ethoxybenzaldehyde (7.5 g) and *p*-ethoxyaniline (6.8 g) were refluxed in boiling ethanol for 30 min to give 4,4'-diethoxybenzylideneaniline, mp 148°C. The Schiff's base (5.06 g) and trichloroacetic acid (3.4 g) were refluxed in 50 ml of tolune for 3 hr. The mixture was washed with 2 *N* HCl and with water, the toluene was distilled off, and the residual oil crystallized from ethanol to give the desired product, mp 105°C, in 65% yield.



The α-trichloromethylbenzylphenyl ethers of Table II were prepared by condensing the appropriate α-trichloromethylbenzyl alcohol (DDT-type carbinol) with equimolar quantities of the appropriate phenol using concentrated sulfuric acid or polyphosphoric acid as the condensing agent. As an example of method A, α-trichloromethyl-*p*-chlorobenzyl alcohol, 5.0 g, was stirred with *p*-chlorophenol, 2.6 g, and sulfuric acid, 35 ml, was added dropwise. After 2 hr of stirring the mixture was poured onto ice and extracted with ether. After drying with sodium sulfate the ether was removed under vacuum and the residue was recrystallized from ethanol to give α-trichloromethyl-*p*-chlorobenzyl *p*-chlorophenyl ether, mp 101°C. In method B, α-trichloromethyl-*p*-methoxybenzyl alcohol, 5.0 g, and *p*-methoxyphenol, 2.48 g, were added to a mixture of phosphorus pentoxide, 18 g, and phosphoric acid, 12 ml, and heated for 1 hr on the steam bath. After standing overnight, ice was added and the mixture extracted with ether. The product was purified by column chromatography on silica gel and eluted with 5% ether in petroleum ether (60–68°C) to give α-trichloromethyl-*p*-methoxybenzyl *p*-methoxyphenyl ether, mp 90°C.

^3H-Labeled α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline "ethoxyaniline" was prepared by the method of Hilton and O'Brien (1964) and purified by column chromatography with silica gel with elution in 8% diethyl ether in petroleum ether (bp 60–68°C). The product has a radiopurity of 99.9+% using solvent systems in Table III with a specific activity of 1.2 mCi per m*M*.

Model Metabolites. For the studies of the metabolism of α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline, "ethoxyaniline," a number of model metabolites were prepared for determinations of R_f by tlc and for detection by the chromo-

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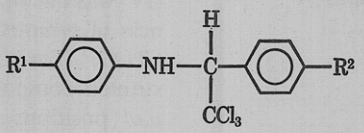
Table I. Chemical Structures and Properties of α-Trichloromethylbenzylanilines



R¹	R²	Schiff's base (I), mp °C	Trichloroaminoethane (II), mp °C	nmr data,[a] δ ppm
Cl	Cl	112	140	α-H 5.07 (s)
CH_3	CH_3	89	60	α-H 5.03 (s), CH_3 2.51 (s), CH_3 2.68 (s)
CH_3O	CH_3O	142	112	α-H 4.96 (s), OCH_3 3.68 (s), OCH_3 3.75 (s)
C_2H_5O	C_2H_5O	148	105	α-H 4.95 (s), OCH_2 3.72–4.17 (m), CH_3 1.2–1.5 (m)
C_2H_5O	CH_3O	124	105	α-H 4.95 (s), OCH_3 3.74 (s), OCH_2 3.71–4.05 (q), CH_3 1.2–1.64 (t)
CH_3O	CH_3	82	60	α-H 5.00 (s), CH_3 2.18 (s), OCH_3 3.7 (s)
CH_3O	Cl	120	80	α-H 4.97 (s), OCH_3 3.67 (s)
Cl	OCH_3	90	98	α-H 4.99 (s), OCH_3 3.7 (s)
CH_3	C_2H_5O	90	62	α-H 5 (s), CH_3 2.18 (s), OCH_2 3.8–4.15 (q), CH_3 1.22–1.49 (t)
C_2H_5O	CH_3	110	84	α-H 4.99 (s), OCH_2 3.66–4.05 (q), CH_3 1.2–1.43 (t), CH_3 2.33 (s)
C_2H_5O	Cl	112	70	α-H 4.97 (s), OCH_2 3.7 (q), CH_3 1.2–1.5 (t)
Cl	C_2H_5O	96	82–83	α-H 5 (s), OCH_2 3.83–4.16 (t), CH_3 1.23–1.53 (q)
C_2H_5O	OCH_2O	121	80	α-H 4.9 (s), OCH_2 3.7–4.02 (q), CH_3 1.2–1.41 (t), OCH_2O 5.9 (s)
2,4-CH_3	CH_3O	67	68	α-H 5.05, CH_3 2.16–2.25 (d), OCH_3 3.73 (s)
3,4-CH_3	CH_3O	65	78–79	α-H 5.0, CH_3 2.08–2.11 (d), OCH_3 3.68 (s)
CH_3O	C_4H_9O	105	65	α-H 4.95, CH_3 0.83–1.07 (m), CH_2CH_2 1.05–1.88 (m), OCH_2 3.8–4.03 (t), OCH_3 3.66
CH_3O	$N(CH_3)_2$	120	105	α-H 4.90 (s), OCH_3 3.66 (s), CH_3 2.9 (s)
NO_2	$N(CH_3)_2$	230	175	α-H 5.07, CH_3 3 (s)
C_2H_5O	H	69	75	α-H 5, OCH_2 3.7–4.05, CH_3 1.2–1.43
C_2H_5O	2-C_2H_5O	60	90	α-H 5.6, OCH_2 3.71–4.26 (m), CH_3 1.2–1.59 (m)
C_2H_5O	C_2H_5O	75	90	α-H 5.05, OCH_2 3.83–4.25 (m), CH_3 1.27–1.6 (m)
C_2H_5O	2,4-C_2H_5O	105	85	α-H 5.6, OCH_2 3.7–4.26 (m), CH_3 1.23–1.56 (m)
C_2H_5O	OH	220	134–135	α-H 5, OCH_2 3.9–4.16 (q), CH_3 1.06–1.5 (t)
OH	C_2H_5O	180	110	α-H 5, OCH_2 3.7–4.1 (q), CH_3 1.2–1.5 (t)

[a] Nmr data: (s) = singlet; (d) = doublet; (t) = triplet; (q) = quartet; (m) = multiplet.

genic reaction with diphenylamine (0.5%) and $ZnCl_2$ (0.5%) in acetone (Kapoor *et al.*, 1970). The two asymmetrical *p*-EtO, *p*-OH α-trichloromethylbenzylanilines and the di-OH derivative were prepared through the Schiff's base reaction previously described. The *p*-ethoxyphenyl dichloromethyl ketone was prepared by dissolving 1 g of "ethoxyaniline" in 10 ml of ethyl alcohol containing 1 g of sodium hydroxide and refluxing for 1 hr. It was purified by column chromatography on silica gel. The *p*-ethoxyaniline and *p*-ethoxybenzoic acid were obtained from Aldrich Chemical Co.

The behavior of these model compounds in selected systems for tlc and their detection on tlc plates are shown in Table III.

Toxicological methods for the determination of the topical LD_{50} values to adult female S_{NAIDM} and R_{SP} houseflies, *Musca domestica* L. and to *Phormia regina*, and LC_{50} values to *Culex pipiens quinquefasciatus* Say, and *Anopheles albimanus* Weid mosquitoes were described by Metcalf *et al.* (1971a). The methods for evaluation of metabolism by mouse liver homogenate and by female R_{SP} housefly and salt-marsh caterpillar larvae *Estigmene acrea* Drury, were described by Kapoor *et*

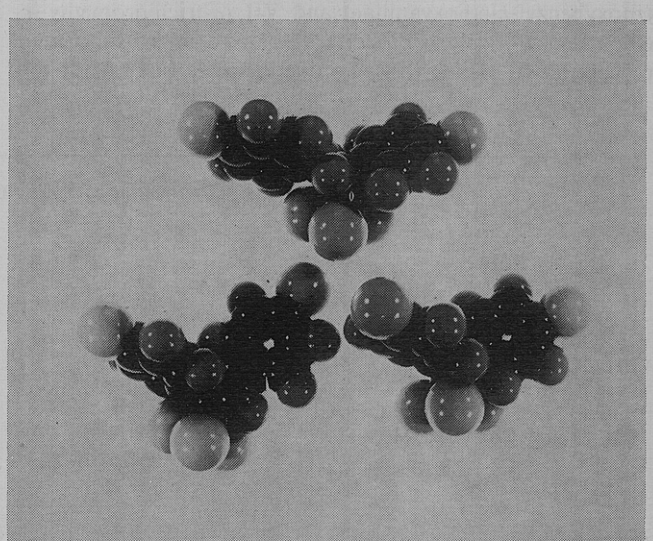
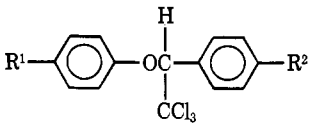


Figure 1. Molecular models of ethoxychlor (top), ethoxyaniline (right), and ethoxyether (left)

Table II. Chemical Structures and Properties of α-Trichloromethylbenzylphenyl Ethers



R¹	R²	mp °C	Method of synthesis	nmr data,[a] δ ppm
Cl	Cl	101	A	α-H 5.4 (s)
CH_3O	CH_3O	90	B	α-H 5.36 (s), OCH_3 3.36 (s), 3.8 (s)
CH_3	CH_3	Liquid	B	α-H 5.41 (s), CH_3 2.33 (s), CH_3 2.83 (s)
C_2H_5O	C_2H_5O	Liquid	B	α-H 5.21 (s), OCH_2 3.61–4.13 (m), CH_3 1.2–1.5 (m)
Cl	CH_3O	90	B	α-H 5.43 (s), OCH_3 3.43 (s)
Cl	C_2H_5O	Liquid	A	α-H 5.43 (s), OCH_2 3.83–4.2 (q), CH_3 1.26–1.5 (t)
CH_3O	C_2H_5O	80	B	α-H 5.43 (s), OCH_2 3.85–4.2 (q), CH_3 1.3–1.5 (t), OCH_3 3.73 (s)
CH_3	C_2H_5O	Liquid	B	α-H 5.32 (s), CH_3 2.16 (s), OCH_2 3.75–4.1 (q), CH_3 1.2–1.46 (t)
CH_3	CH_3O	142	B	α-H 5.62, CH_3 2.33 (s), OCH_3 3.83 (s)

al. (1970). The techniques for model ecosystem evaluation were described by Metcalf *et al.* (1971b).

RESULTS AND DISCUSSION

Insect Toxicity of Anilines. The data of Table IV indicate the insect toxicity of 27 α-trichloromethylbenzylanilines to *Musca domestica* (S_{NAIDM} and R_{SP} strains), *Phormia regina*, and to *Culex fatigans* and *Anopheles albimanus*. The most effective insecticide was XI(α-trichloromethyl-*p*-ethoxybenzyl-*p*-methylaniline), which had the lowest LD_{50} values to both strains of housefly and to *Phormia* and was only slightly less effective than I and VII to the mosquito larvae. This compound also had the lowest synergized LD_{50} values, with piperonyl butoxide, to the test insects. Compounds which were only slightly less effective included III (α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline) and VII (α-trichloromethyl-*p*-ethoxybenzyl-*p*-chloroaniline). The *p,p'*-dichloro-substituted compound (I) was a very effective larvicide but was of low toxicity to adult insects. Although the *p,p'*-dimethoxy derivative (II) was of very low activity, the *p,p'*-dimethyl derivative (IV) was one of the better compounds. The order of effectiveness of symmetrical substitution was $C_2H_5O > CH_3 > Cl > CH_3O$. Toxicity was greatly decreased in the monosubstituted compound (XIX) or by substitution in the *o,p'* or *p,o'* positions (XX, XXI). Toxicity was substantially decreased by substitution of either aniline or benzyl ring with C_4H_9O (XVIII), $N(CH_3)_2$ (XXV), O_2N (XXVI), or cyclohexyl (XXVII), or by 2,4-disubstitution (XXII, XXIV) or 3,4-disubstitution (XXIII).

Insect Toxicity of Ethers. The data of Table V indicate the insect toxicity of nine α-trichloromethylbenzyl phenyl ethers. The most effective insecticide was XXXIII (α-trichloromethyl-*p*-ethoxybenzyl *p*-chlorophenyl ether) which had the lowest LD_{50} values to the housefly and was only slightly less toxic to *Phormia* than the *p,p'*-diethoxy compound XXXI and to mosquito larvae than XXVIII. Toxicity in the symmetrical substituents was in the general order of $C_2H_5O > Cl > CH_3O > CH_3$. In general the ethers were less toxic than the corresponding anilines.

Insect Toxicity of Thioether. The α-trichloromethyl-*p*-methoxybenzyl *p*-chlorophenylthioether (XXXVII) shown in Table V was only about 0.1 times as effective as the corresponding benzyl phenyl ether (XXXII). This compound can readily assume a configuration similar to DDT as shown by molecular models. However, the ready oxidation to sulfoxide and sulfone should substantially interfere with its appropriate configuration and polarity. It is synergized to a high degree, suggesting therefore that its lack of toxicity may result from thioether oxidation *in vivo*.

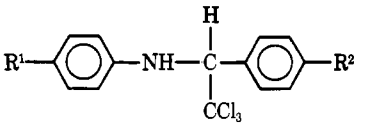
Synergistic Ratios for Flies. The synergistic ratios or SR values (LD_{50} alone/LD_{50} synergized with piperonyl butoxide) shown in Tables IV and V indicate the role of the mixed function oxidase (MFO) in detoxifying the individual compounds. The synergized LD_{50} values express the intrinsic toxicity of the compounds (Metcalf *et al.*, 1971a). For the α-trichloromethylbenzylanilines, the compounds with highest intrinsic toxicity are CH_3O, C_2H_5 (XI), C_2H_5O, OCH_3 (XIV), C_2H_5O, Cl (VIII), and C_2H_5O, OC_2H_5 (III). Compound XI was also outstandingly toxic to *Phormia*, which is deficient in MFO (Metcalf *et al.*, 1971a). The CH_3O, OCH_3 compound (II) had the highest SR values for susceptible and resistant flies (37–100) and was also substantially synergized in *Phormia*, indicating rapid detoxication, while the Cl, OC_2H_5 compound (VII) has the lowest SR values (1.8–2.3). In general higher SR values were found with compounds having a CH_3O group on the ring adjacent to the –NH– linkage (VI, X). This suggests that the

Table III. Properties of "Ethoxyaniline" and Its Model Metabolites

Compound	mp °C	Thin-layer chromatography (R_f)[a] Sol 1	Sol 2	Detection[b] uv	Chromogenic
$C_2H_5OC_6H_4NHCH(CCl_3)C_6H_4OC_2H_5$	105	0.34	0.71	Brown	Yellow
$C_2H_5OC_6H_4C(O)CHCl_2$	Liquid	0.4	0.70	Light brown	None
$C_2H_5OC_6H_4NHCH(CCl_3)C_6H_4OH$	134–5	...	0.44	Light yellow	Yellow
$HOC_6H_4NHCH(CCl_3)C_6H_4OC_2H_5$	110	...	0.4	Light yellow	Yellow
$HOC_6H_4NHCH(CCl_3)C_6H_4OH$	110	...	0.4	Yellow	Yellow
$C_2H_5OC_6H_4NH_2$	bp 250	...	0.36	Brown	None
$C_2H_5OC_6H_4COOH$	197–9	...	0.2	None	None

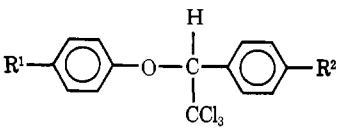
[a] Sol 1, ether–petroleum ether (60–68 °C) 1:9; Sol 2, ether–petroleum ether 1:1. [b] Chromogenic reagent: diphenylamine 0.5% plus zinc chloride 0.5% in acetone; spray and heat at 110 °C for 10 min.

Table IV. Toxicity of α-Trichloromethylbenzylanilines to Insects



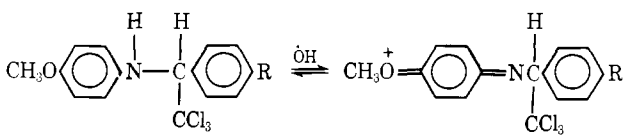
	Substituents		Topical LD_{50} μg per g for *Musca domestica*						*Phormia regina*			LC_{50} ppm	
			S_{NAIDM}			R_{SP}						*Culex fatigans* larvae, ppm	*Anopheles albimanus* larvae, ppm
	R^1	R^2	Alone	with pb	SR	Alone	with pb	SR	Alone	with pb	SR		
I	Cl	Cl	115	13.5	8.5	220	25	8.8	>250	42.5	>5.5	0.026	0.090
II	CH_3O	CH_3O	>500	5.5	>100	500	13.5	>37	117.5	35.0	3.4	>1.0	0.64
III	C_2H_5O	C_2H_5O	15.5	4.2	3.7	20.5	6.5	3.2	33.7	9.7	3.7	0.19	0.28
IV	CH_3	CH_3	43.0	5.0	8.6	43.0	6.5	6.6	33.7	21.0	1.6	0.18	0.12
V	Cl	CH_3O	23.0	10.5	2.2	35.5	12.0	3.0	55.0	15.2	3.6	0.068	0.12
VI	CH_3O	Cl	39.0	6.5	6.0	49.0	14.0	3.4	127.5	80	1.6	0.44	0.37
VII	Cl	C_2H_5O	19.0	10.5	1.8	31.0	13.5	2.3	24.5	20.0	1.2	0.032	0.060
VIII	C_2H_5O	Cl	19.5	4.1	4.8	45.0	13.0	3.5	37.5	13.2	2.8	0.22	0.26
IX	CH_3	CH_3O	35.0	8.5	4.1	50.0	12.0	4.5	16.5	11.50	1.4	0.26	0.28
X	CH_3O	CH_3	48.0	8.5	5.6	65.0	7.5	8.7	135	14.0	9.6	0.54	0.48
XI	CH_3	C_2H_5O	12.5	2.5	5.0	14.0	4.4	3.2	7.7	4.2	1.8	0.073	0.085
XII	C_2H_5O	CH_3	35.0	14.5	2.4	70.0	19.5	3.6	95.0	42.5	2.2	0.33	0.30
XIII	CH_3O	C_2H_5O	23.5	5.5	4.2	41.0	12.5	3.3	22.0	12.5	1.8	0.42	0.68
XIV	C_2H_5O	CH_3O	30.0	3.9	7.7	37.0	12.0	3.1	112.5	20.0	5.6	0.34	1.0
XV	Cl	CH_3	90.0	21.0	4.3	500	36.0	14	72.5	62.5	1.1	0.066	0.13
XVI	CH_3	Cl	65.0	16	4.1	ca. 125	30	4.2	36.25	25	1.45	0.18	0.14
XVII	C_2H_5O	OCH_2O	19.5	3.8	5.1	35.5	11.0	3.2	>250	32.5	>7.7	0.23	0.25
XVIII	CH_3O	C_4H_9O	370	12.5	30	230	32.0	7.2	>250	67.5	>4	0.60	0.77
XIX	C_2H_5O	H	>500	31.0	>16	>500	120	>4	>250	>250	...	>1.0	0.61
XX	4-C_2H_5O	2-C_2H_5O	>500	500	...	>500	>500	...	>250	>250	...	>1.0	>1.0
XXI	2-C_2H_5O	4-C_2H_5O	>500	110	>5	>500	140	>3.5	>250	>250	...	1.0	>1.0
XXII	2-C_2H_5O	2,4-C_2H_5O	>500	500	...	>500	500	...	>250	>250	...	>1.0	>1.0
XXIII	3,4-CH_3	CH_3O	85.0	6.5	13.0	100	28.0	3.6	77.5	15.5	5.0	0.30	0.18
XXIV	2,4-CH_3	CH_3O	95.0	14.0	6.8	140	29.0	4.8	97.5	25.0	3.9	0.28	0.080
XXV	CH_3O	$N(CH_3)_2$	310	16.0	19.4	>500	13.5	>37	>250	85.0	>3	0.70	1.0
XXVI	O_2N	$N(CH_3)_2$	>500	120	>4	>500	500	...	>250	>250	...	0.38	>1.0
XXVII	C_6H_{11}	CH_3O	>500	48	>10	>500	165	>3	>250	112.5	>2	1.0	>1.0

Table V. Toxicity of α-Trichloromethylbenzylphenyl Ethers to Insects



	Substituents		Topical LD_{50} μg per g for *Musca domestica*						*Phormia regina*			LC_{50} ppm	
			S_{NAIDM}			R_{SP}						*Culex fatigans* larvae	*Anopheles albimanus* larvae
	R^1	R^2	Alone	pb	SR	Alone	pb	SR	Alone	pb	SR		
XXVIII	Cl	Cl	90	90	1.0	>500	180	3.6	>250	>250	...	0.035	0.014
XXIX	CH_3O	CH_3O	300	14.0	22	>500	57.5	>8.7	125	82.5	1.5	0.51	0.10
XXX	CH_3	CH_3	265	90	2.9	>500	145	>3	135	100	1.35	0.12	0.18
XXXI	C_2H_5O	C_2H_5O	27.0	13.0	2.1	42	24.5	1.7	16.5	16.5	1.0	0.11	0.07
XXXII	Cl	CH_3O	107.5	17.0	6.3	130	82.5	1.6	205	115	1.8	0.14	0.038
XXXIII	Cl	C_2H_5O	18.5	9.5	1.9	31	12.5	2.5	31.2	31.2	1.0	0.067	0.044
XXXIV	CH_3O	C_2H_5O	45.0	5.0	9.0	90	14.0	6.4	30.0	16.0	1.9	0.14	0.034
XXXV	CH_3	C_2H_5O	72.5	20.5	3.5	65	22.0	2.9	46.2	30.0	1.5	0.18	0.066
XXXVI	CH_3	CH_3O	>500	39.0	>13	>500	135	>4	>250	>250	...	0.65	>1.0
XXXVII	$ClC_6H_4SCH(CCl_3)C_6H_4OCH_3$		>500	110	>5	>500	225	>2	...	...	...	0.72	>1.0

anilinium structure favors attack by MFO·OH radical on the positively polarized CH_3O.



The CH_3O, $N(CH_3)_2$ compound (XXV) also had a high SR value, suggesting MFO attack by *N*-dealkylation.

For the α-trichloromethylbenzylphenyl ethers (Table V), the compounds with the highest intrinsic toxicity were CH_3O, OC_2H_5 (XXXIV), Cl, OC_2H_5 (XXXIII), and C_2H_5O, OC_2H_5 (XXXI). The CH_3O, OCH_3 compound (XXIX) had the largest SR value in both susceptible and resistant houseflies (>8.7–22), indicating rapid detoxication, while the Cl, OC_2H_5 compound (XXXIII) had the lowest SR values (1.9–2.5), in good agreement with the corresponding anilines. Comparisons of the combined effects of synergism in all three series of com-

Table VI. Comparison of Insecticidal Activity of Symmetrical DDT-Type Ethanes, Anilines, and Ethers

	LD_{50} μg per g		LD_{50} ppm	
	Musca	*Phormia*	*Culex*	*Anopheles*
p,p'-Substituted diphenyltrichloroethanes				
Cl	14.0	11.5	0.07	0.015
CH_3	100	61.2	0.08	0.17
CH_3O	45	10.0	0.067	0.18
C_2H_5O	7.0	6.9	0.04	0.086
p,p'-Substituted α-trichloromethylbenzylanilines				
Cl	115	>250	0.026	0.090
CH_3	43	33.7	0.18	0.12
CH_3O	>500	117.5	>1.0	0.64
C_2H_5O	15.5	35.2	0.19	0.24
p,p'-Substituted α-trichloromethylbenzyl phenyl ethers				
Cl	90	>250	0.035	0.014
CH_3	265	135	0.12	0.18
CH_3O	300	125	0.51	0.10
C_2H_5O	27.0	16.5	0.11	0.07

pounds (Metcalf *et al.*, 1971a) suggests that *O*-demethylation is the most efficient detoxication process for DDT-type compounds and that the rates in which this process occurs in houseflies are relatively independent of the types of linkage between the two aryl rings, *e.g.*, –C–, –NC, or –OC–.

Toxicity to DDT-Resistant Flies. Comparison of the LD_{50} values in the present work with those for DDT analogs determined under identical conditions (Metcalf *et al.*, 1971a) affords an indication of the relative effectiveness of the trichloroethanes, trichloromethylbenzylanilines, and trichloromethylbenzylphenyl ethers to the DDT-resistant R_{SP} housefly. This strain is presently maintained at a moderate level of DDT resistance, topical LD_{50} 170 μg per g, compared with 14 μg per g for the S_{NAIDM} (22°C), giving a resistance ratio or RR of 12. For the effective α-trichloromethylbenzylanilines, RR values for the two strains range from 1.0 to 2.3 (Table IV). For the effective α-trichloromethylbenzylphenyl ethers, RR values range from 1.2 to 2.0 (Table V). The substantially greater effectiveness of both α-trichloromethylbenzylanilines and α-trichloromethylbenzylphenyl ethers to the DDT-resistant R_{SP} flies is very probably due to the unsuitability of these compounds as substrates for DDT'ase.

Stereochemistry of Active Compounds. The data in Tables IV and V, together with previous study of insecticidal activity of DDT analogs (Metcalf and Fukuto, 1968; Metcalf *et al.*, 1971a), provide indications of the optimum molecular geometry associated with DDT-like action. It is evident from Figure 1 that the diphenyltrichloroethanes, α-trichloromethylbenzylanilines, and α-trichloromethylbenzylphenyl ethers can form "molecular wedges" with nearly identical size and shape capable of interaction with the membrane pores (Mullins, 1956) or sodium gates (Holan, 1969) of the nerve axon, or whatever lipoprotein site is presumed to be the DDT receptor (Gunther *et al.*, 1954). As shown in Table VI, the *p,p'*-diethoxy-substituted members of each series are outstandingly active and the LD_{50} and LC_{50} values for these compounds fall within a fivefold range. This suggests that the DDT receptor site is optimally shaped to accommodate a molecule with dimensions close to those of "ethoxychlor" (Holan, 1971).

Metabolism of Ethoxyaniline. The compound α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline was selected as one of the most active insecticides (Table IV) for investigation of metabolism in the adult female housefly, in the larval salt-marsh caterpillar *Estigmene acrea* Drury, and by mouse liver homogenate. Table VII shows the results of incubation for 1 hr of 0.01 μ*M* ^3H-labeled compound with a mouse liver homogenate prepared as described by Kapoor *et al.* (1970), fortified with NADPH and nicotinamide. Under these conditions the compound was attacked by *O*-dealkylation to produce either one or both of the monophenolic derivatives, and the compound was also cleaved at the C–N bond to form *p*-ethoxyaniline. Topical application of 1.0 μg of ^3H-ethoxyaniline to the prothorax of the female R_{SP} housefly resulted in recovery after 24 hr of 94.2% of the ^3H, as excreta, 10%, body homogenate, 65%, and body wash, 19.2%. The percentage of various ^3H metabolites in homogenate and excreta is shown in Table VII. Similar experiments were performed by feeding 0.5 mg of ^3H-ethoxyaniline to 4th instar salt-marsh caterpillar larvae where, after 24 hr, 75% of the ^3H was recovered in excreta and the remainder in the body homogenate, with the metabolic distribution shown in Table VII. The experiments in insect metabolism show a picture similar to that of the mouse liver homogenate. Ethoxyaniline is metabolized in both insect and mammal by O-dealkylation to form both mono-

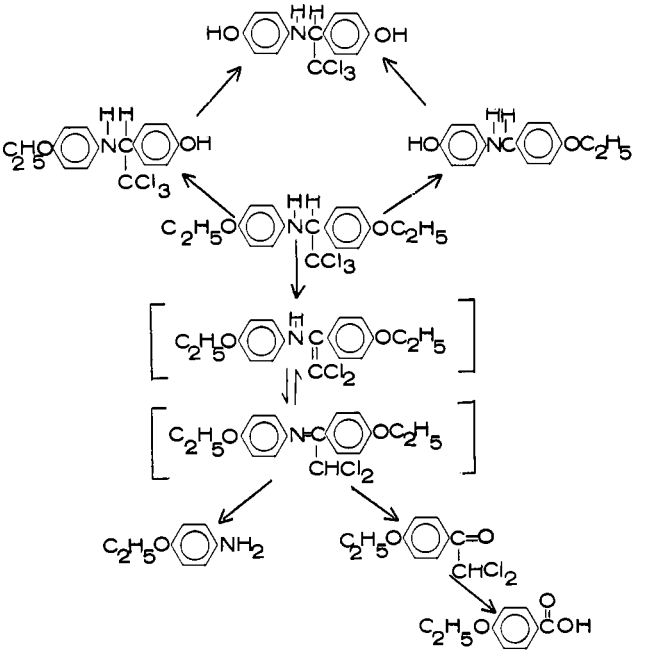


Figure 2. Metabolism of ethoxyaniline

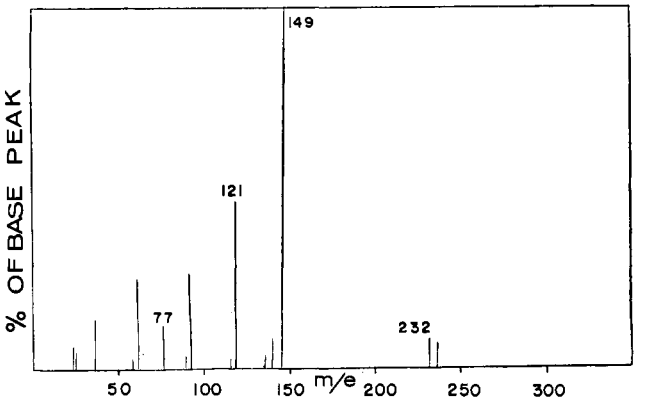


Figure 3. Mass spectrum of *p*-ethoxydichloroacetophenone, a metabolite of ethoxyaniline

Table VII. Metabolism of α-Trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline by Housefly, Salt-Marsh Caterpillar, and Mouse Liver Homogenate

Compound	Female housefly, percent ^3H in		Salt-marsh caterpillar, percent ^3H in		Mouse liver, percent ^3H in homogenate
	Homogenate	Excreta	Homogenate	Excreta	
$C_2H_5OC_6H_4NHCH(CCl_3)C_6H_4OC_2H_5$	37.6	22.9	9.0	2.5	30
$C_2H_5OC_6H_4NHCH(CCl_3)C_6H_4OH$ or $HOC_6H_4NHCH(CCl_3)C_6H_4OC_2H_5$	11.5	25.5	14.4	4.1	7
$HOC_6H_4NHCH(CCl_3)C_6H_4OH$	8.4	5.7	...	...	...
$C_2H_5OC_6H_4C(O)CHCl_2$	10.4	9.7	8.7	3.1	...
$C_2H_5OC_6H_4NH_2$	8.3	9.1	15.0	8.4	13.7
Unknown I	14.3	11.1	11.5	...	20
Conjugates	15.8	9.3	40	81	29

Table VIII. Distribution of ^3H-"Ethoxyaniline" and its Metabolites in a Model Ecosystem

	Concentration (ppm) of ethoxyaniline equivalents				
	H_2O	*Oedogonium* (algae)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total ^3H	0.363	3.03	36.0	1.0	0.30
$C_2H_5OC_6H_4NHCH(CCl_3)C_6H_4OC_2H_5$	0.055	1.09	22.68	0.28	0.04
$C_2H_5OC_6H_4NHCH(CCl_3)C_6H_4OH$	0.031	0.43	2.02	0.13	0.08
$HOC_6H_4NHCH(CCl_3)C_6H_4OH$	...	...	1.512	...	0.03
$C_2H_5OC_6H_4C(O)CHCl_2$	...	0.3	3.542	...	0.28
$C_2H_5OC_6H_4NH_2$	0.053	0.42	2.556	0.01	0.03
$C_2H_5OC_6H_4C(O)OH$	0.123	0.38	1.800	0.06	0.03
Unknown R_f 0.4	0.053	...	...	0.15	...
Conjugates, polar metabolites	0.042	0.40	2.520	0.22	0.06

and bis-phenols and the compound is also cleaved at the C–N bond to form *p*-ethoxyaniline and *p*-ethoxyphenyldichloromethylketone. Relatively large amounts of polar conjugates of the phenols were also found. The proposed metabolic pathways are shown in Figure 2.

The *p*-ethoxydichloroacetophenone was characterized by tlc (Table III). Its nmr spectrum showed δ(OCH_2) 3.72–4.17, (CH_3) 1.2–1.5, (H) 6.68. Mass spectrometry showed a fragmentation pattern of a typical aromatic ketone. The peak at mass 232 was that of the basic molecule, which in turn gave rise to a fragment at *m/e* 149, $C_2H_5OC_6H_4C{\equiv}O^+$. This base peak underwent O-dealkylation to produce the $HOC_6H_4C{\equiv}O^+$ ion, which fragmented further to give phenyl ion at *m/e* 77 (Figure 3). Identities of α-trichloromethyl-*p*-ethoxyphenyl-*p*-hydroxyaniline, α-trichloromethyl-*p*-hydroxybenzyl-*p*-ethoxyaniline, and α-trichloromethyl-*p*-hydroxybenzyl-*p*-hydroxyaniline were confirmed by tlc cochromatography with model metabolites and by the characteristic yellow color which appeared upon exposure to ultraviolet light (Kapoor *et al.*, 1970).

Metabolism in a Model Ecosystem. The environmental fate of any new pesticide is a factor of paramount importance in determining how and where it might be used. The behavior of ^3H-α-trichloromethyl-*p*-ethoxybenzyl-*p*-ethoxyaniline has been studied by the model ecosystem technique following application of 5.0 mg of labeled compound to *Sorghum* plants (Metcalf *et al.*, 1971b). A summary of the results is shown in Table VIII. The environmental metabolites from the organisms in the model ecosystem after 33 days were identified by cochromatography with standards of known constitution, by appropriate chromogenic reagents (Table III), and by high-resolution mass spectrometry. The data of Table VIII indicate the O-dealkylation of ethoxyaniline at both *p*-ethoxybenzyl and *p*-ethoxyaniline moieties to form the mono- and bis-phenols, as shown in Figure 2. The most interesting biological reaction is the result of dehydrochlorination to form the apparently transitory dichloroethylene intermediate which undergoes a tautomeric shift to form the α-dichloromethyl-*p*-ethoxybenzylidine-*p*-ethoxyaniline. The latter compound is readily hydrolyzed to *p*-ethoxyaniline and *p*-ethoxydichloroacetophenone, which subsequently forms *p*-ethoxybenzoic acid (Figure 2).

It is of interest to compare the model ecosystem behavior of the "ethoxyaniline" (Table VIII) with the corresponding DDT analog "ethoxychlor" or 2,2-bis(*p*-ethoxyphenyl)-1,1,1-trichloroethane, as evaluated by Kapoor *et al.* (1972). Ethoxychlor was found in the snail to 58.6 ppm and in the fish to 0.92 ppm, along with its ethylene and the mono- and bis-phenols formed by O-dealkylation. The ecological magnification of ethoxychlor from water to fish was 1500-fold as compared with 0.7-fold for the ethoxyaniline. It appears that both compounds are substantially biodegradable, but that the opportunity for metabolic cleavage between the C–N bond of the anilines enhances the biodegradability. Comparing the Biodegradability Indices, which we have defined as polar metabolites/nonpolar metabolites in the fish, the values are ethoxychlor 0.44 and ethoxyaniline 0.12. Clearly, both compounds are substantially more biodegradable than DDT which, in identical experiments, was concentration from water to fish 84,000-fold and had a Biodegradability Index of 0.015.

ACKNOWLEDGMENT

We gratefully acknowledge the skillful assistance of Penny Hansen and Ruth Millholin in performing the bioassays.

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Received for review January 27, 1972. Accepted March 10, 1972. Supported in part by grants from the Rockefeller Foundation and Herman Frasch Foundation.

Synthesis of 2-Acyl-3-amino-1,2,4-triazoles and 2-Acetyl-3-amino-1,2,4-triazole-5-^{14}C

Melvin Look* and Larry R. White

Acylation of 3-amino-1,2,4-triazole (amitrole) with *N*-acylimidazoles gave 2-acyl-3-amino-1,2,4-triazoles exclusively, whereas previous methods using acyl halides or anhydrides have yielded mixtures of acylated and diacylated derivatives. The new method was used to synthesize 2-acetyl-3-amino-1,2,4-triazole-5-^{14}C.

The acylation of 3-amino-1,2,4-triazole (amitrole, I) with acetyl chloride or acetic anhydride yields mixtures of acetylated and diacetylated derivatives (Staab and Seel, 1959; Van Den Bos, 1960; Coburn *et al.*, 1970). In studies of systemic pesticides at the Pacific Southwest Forest and Range Experiment Station, we needed pure, acylated carbon-14-labeled amitrole. Previous methods were unsatisfactory because of low yields and difficulties in the separation of products. We found that *N*-acetylimidazole (II) acylates amitrole to 2-acetyl-3-amino-1,2,4-triazole (III) without the formation of other isomers, even with a large excess of acetylating agent (Figure 1).

By using 3-amino-1,2,4-triazole-5-^{14}C, radiopurity was maintained. The imidazole method was extended to the synthesis of higher acylated amitroles (Table I).

EXPERIMENTAL

N-Acetylimidazole was purchased from Pierce Chemical Co., Rockford, Ill. The higher analogs were synthesized from acyl halide and imidazole by using the procedure of Staab (1962). Amitrole was obtained from the American Cyanamid Co., Wayne, N.Y. 3-Amino-1,2,4-triazole-5-^{14}C was purchased from New England Nuclear, Boston, Mass., and was used without further purification. Microanalyses were performed by the Microchemical Laboratory of the University of California, Berkeley. Nmr spectra were made in dimethyl sulfoxide-d_6 solvent with an internally locked (TMS) Varian HR-100.

2-Acetyl-3-amino-1,2,4-triazole and Higher Analogs. Amitrole (0.50 g, 5.94 mmol) and 2.60 g (23.61 mmol) of acetylimidazole in 25 ml of acetonitrile were heated under reflux for 2 hr. Dilution with 100 ml of water, extraction of the resulting solution with methylene chloride, and evaporation of the solvent gave, after recrystallization from toluene, 0.45 g (60%) of 2-acetyl-3-amino-1,2,4-triazole, mp 153° C (Van Den Bos had reported mp at 151–154° C). The nmr chemical shift of carbon-5 (3) proton agrees with that in the literature (Coburn *et al.*, 1970). Procedures for higher analogs were also identical, except that stoichiometric amounts of reagents were used to preclude large amounts of difficult-to-remove higher fatty acids.

2-Acetyl-3-amino-1,2,4-triazole-5-^{14}C. 3-Amino-1,2,4-triazole-5-^{14}C (6.9 mg, 98.4 μCi at 1.2 mCi/mmol) was heated under reflux with 36.1 mg (0.33 mmol) of acetylimidazole in 5 ml of acetonitrile for 3 hr. The reaction mixture was concentrated in a nitrogen stream and was purified by preparative thin-layer chromatography on silica gel G by using ethyl acetate–acetone (1:2) solvent (R_f = 0.77). The band detected by X-ray autoradiogram was removed and extracted with acetone. Evaporation gave a solid containing 6.9 mg, 65.3 μCi at 1.2 mCi/mmol (66% radioactive yield), of acetylamitrole.

Table I. Synthesis of 2-Acyl-3-amino-1,2,4-triazoles

Compound	Melting point, °C	Chemical shifts of carbon-3 proton δ C–H (ppm)	Analysis calcd: found, %
2-Acetyl-3-amino-1,2,4-triazole	153	7.53[a]	Known
2-Hexanoyl-3-amino-1,2,4-triazole	80–81	7.47	C, 52.73; C, 52.54 H, 7.74; H, 7.87
2-Nonanoyl-3-amino-1,2,4-triazole	74–75	7.48	C, 58.90; C, 58.56 H, 8.99; H, 8.93
2-Decanoyl-3-amino-1,2,4-triazole	82–83	7.48	C, 60.48; C, 61.00 H, 9.30; H, 9.47
2-Dodecanoyl-3-amino-1,2,4-triazole	87–88	7.45	C, 63.13; C, 63.37 m, 9.84; H, 10.09

[a] 7.57 according to Coburn *et al.* (1970).

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