Selective Toxicity of Isopropyl Parathion

Effect of Structure on Toxicity and Anticholinesterase Activity

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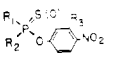
Isopropyl parathion and a series of related phosphate, phosphonate, and phosphinate esters containing branched alkyl groups were examined for toxicity to the housefly, honey bee, and white mouse. Anticholinesterase activity and alkaline hydrolysis rates of the PO esters were also determined. Isopropyl parathion and isopropyl paraoxon were found to be >212- and 17.8-fold more toxic to the housefly than to the honey bee, respectively; both were somewhat less toxic to the white mouse than the corresponding dimethyl and diethyl analogs. A number of other branched alkyl *p*-nitrophenyl phosphorothioate and phosphonothioate esters also showed selective action, particularly *O*-ethyl *O*-*p*-nitrophenyl isopropylphosphonothioate, which was >39-fold more toxic to the housefly than honey bee. However, the order of toxicity was reversed with the corresponding PO analog. Substantial differences were found in the rates of inhibition of cholinesterase from the two insects by some of the compounds and in the case of isopropyl paraoxon there was reasonable agreement between anticholinesterase activity and toxicity. The over-all data indicate that the selectivity of these esters may be explained only in part by differences in cholinesterase inhibition and that other factors also are involved in their selective action.

Metcalf and March's observation (1949), that isopropyl parathion, *O,O*-diisopropyl *O*-*p*-nitrophenyl phosphorothioate, is approximately 250 times more toxic to the housefly, *Musca domestica* (LD_{50} 4.2 μg. per gram) than to the honey bee, *Apis mellifera* (LD_{50} >1000 μg. per gram), has stimulated other recent studies on this remarkable example of selective toxicity in insects (Dauterman and O'Brien, 1964; Metcalf and Frederickson, 1965). Since *O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate (parathion) and the corresponding dimethyl ester (methyl parathion) are equally toxic to the housefly and honey bee, the unusual selective action of isopropyl parathion presents a fascinating problem owing to the minor difference in structure of these compounds. Honey bees generally are sensitive to organophosphorus and carbamate insecticides and the low toxicity of isopropyl parathion is of considerable academic interest from a toxicological viewpoint and has practical significance because of the economic importance of bees and hymenoptera in general.

Metcalf and Frederickson (1965) examined a large series of substituted phenyl dialkyl phosphorothionates and phosphates for toxicity to houseflies and honey bees and found that the diisopropyl esters were considerably less toxic to bees than to flies despite significant alterations in the substituted phenyl moiety. In comparison the dimethyl, diethyl, and dipropyl analogs were all nearly equitoxic to both species. These findings clearly indicated that the difference in toxicity of diisopropyl phosphorothionate esters to honey bees and houseflies is in some way related to the branched isopropyl moiety, either by interfering with the activation of P=S to P=O or by reducing inhibition of honey bee cholinesterase.

This paper is concerned with the relationship between the anticholinesterase activity of phosphate, phosphonate, and phosphinate analogs of isopropyl parathion and the toxicity of these materials and their sulfur analogs to the honey bee, housefly, and white mouse. In particular, an attempt is made to assess the effect of branching in R_1 or R_2 where the two groups are alkoxy or alkyl moieties, and R_3 is hydrogen or methyl.



MATERIALS AND METHODS

The various intermediate phosphoro-, phosphino-, and phosphonochloridates, and their sulfur analogs were prepared as described below. In most cases the formation of small amounts of impurities was unavoidable; therefore, the products were not characterized but were carried on to the corresponding *p*-nitrophenyl esters. *O,O*-Diisopropyl phosphorochloridothioate [b.p. 78–82° C. (3.0 mm.), n_D^{25} 1.4588] was prepared according to Fletcher *et al.* (1950). *O*-Methyl *O*-isopropyl phosphorochloridothioate [b.p. 32–35° C. (1.25 mm.), n_D^{25} 1.4599] and *O*-ethyl *O*-isopropyl phosphorochloridothioate [b.p. 44–46° C. (1.75 mm.), n_D^{25} 1.4635] were prepared according to Melnikov *et al.* (1956) from isopropyl phosphorothioic dichloride. Diisopropyl phosphorochloridate [b.p. 51–55° C. (1.0 mm.), n_D^{25} 1.4141] was prepared from triisopropyl phosphite (McCombie *et al.*, 1945). Isopropyl isopropylphosphonochloridate [b.p. 50–51° C. (4.5 mm.), n_D^{25} 1.4260] was prepared from isopropylphosphonic dichloride (Clay, 1956) by treatment with one equivalent of isopropyl alcohol and pyridine in *n*-hexane. Ethyl isopropylphosphonochloridate [b.p. 84–87° C. (10 mm.), n_D^{25} 1.4335] was prepared from diethyl isopropylphosphonate (Fukuto and Metcalf, 1959). Isopropyl isopropylphosphonochloridothionate [b.p. 48–51° C. (3.25 mm.) n_D^{25} 1.4836],

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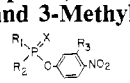
ethyl isopropylphosphonochloridothionate [b.p. 51–54° C. (2.75 mm.), n_D^{25} 1.4859], and isopropyl ethylphosphonochloridothionate [b.p. 44–50° C. (1.25 mm.), n_D^{25} 1.4611] were prepared from the respective alkylphosphonothioic dichloride according to Razumov *et al.* (1952) and Hoffman *et al.* (1958). Methyl *tert*-butylphosphonochloridate [b.p. 46–48° C. (3.25 mm.), n_D^{25} 1.4412] was prepared from *tert*-butylphosphonic dichloride (Clay, 1956) by treatment with an equivalent amount of sodium methoxide. Analysis for chlorine gave 20.87%, calculated for $C_5H_{12}ClO_2P$, Cl = 20.82%. Dipropylphosphinothioic chloride [b.p. 100–02° C. (4 mm.)] was prepared from dipropylphosphinic chloride (Mastryukova *et al.*, 1959) by treatment with phosphorus pentasulfide. Diisopropylphosphinothioic chloride was prepared according to Christen *et al.* (1959) [b.p. 75–77° C. (1.75 mm.), n_D^{25} 1.5245].

The various phosphate, phosphonate, and phosphinate esters of 4-nitrophenol and 3-methylnitrophenol listed in Table I were prepared in the usual manner from the sodium salt of the phenol and the respective chloridate (Fukuto and Metcalf, 1959; Hollingworth *et al.*, 1967) using toluene or 2-butanone as the solvent. The synthesis of compounds XII, XV, and XVIII was reported in previous papers from this laboratory (Fukuto and Metcalf, 1959; Fukuto *et al.*, 1961). Compound V, *O*-methyl *O*-isopropyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate, could not be obtained pure by falling-film distillation and was purified on Woelm acid-alumina column (anionotropic, activity grade 1 for chromatographic analysis) by elution with benzene. Elemental analyses reported in Table I for this compound were obtained from column-purified material.

Methods for the determination of toxicity to houseflies have been described (Metcalf and March, 1949). A susceptible strain, S_{NAIDM}, of female housefly was used and mortality was estimated 24 hours after treatment. Toxicity to the honey bee was determined in a similar manner after application of the test compound on the dorsal surface of the thorax following anesthetization with carbon dioxide. Following treatment, the bees were placed in 5³-inch wire cages containing a feeding vial filled with 40% honey water. The treated bees were held in a 60° F. room and the mortality was estimated after 24 hours. In a number of cases the bees were held in an 80° F. room for comparative purposes. Generally, two replicates of 20 bees per replicate (40 bees) were treated at each dosage level on three different days. Mammalian toxicity was determined orally on 3- to 6-month-old Swiss white mice reared from a strain originally purchased from Curd's Caviary, La Puente, Calif. The test compound was dissolved in olive oil and applied as previously described (Hollingworth *et al.*, 1967). Mortalities were recorded after 24 hours.

The techniques for the preparation of fly head cholinesterase and determination of anticholinesterase activity (I_{50} values) have been described (Fukuto and Metcalf, 1956). The incubation time for inhibitor and cholinesterase was 20 minutes. The preparation and determination of the I_{50} values of bee head cholinesterase were carried out in the same manner. Affinity constants (K_i) and phosphorylation rate constants (k_p) were determined for the cholinesterase and isopropyl paraoxon (diisopropyl 4-nitrophenyl phosphate) according to a procedure previously described (Hollingworth *et al.*, 1967; Main, 1964). Fly or bee head cholinesterase solutions were prepared in pH 7.6, 4 m*M* phosphate buffer. The activities of the enzyme preparations were 0.83 μM AChBr hydrolyzed per min-

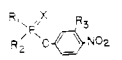
Table I. Physical Properties of Phosphate, Phosphonate, and Phosphinate Esters of 4-Nitrophenol and 3-Methyl-4-nitrophenol



	R_1	R_2	R_3	X	B.p., °C.[a] (Mm.)	n_D^{25}	Analysis			
							Calcd.		Found	
							C	H	C	H
I	i-C_3H_7O	i-C_3H_7O	H	S	57–58[b]	—	45.14	5.68	45.41	6.14
II	i-C_3H_7O	i-C_3H_7O	H	O	143(0.02)	1.5038	47.53	5.98	47.38	6.40
III	i-C_3H_7O	i-C_3H_7O	CH_3	S	49–51[b]	—	46.84	6.05	46.07	6.18
IV	CH_3O	i-C_3H_7O	H	S	58–60[b]	—	41.24	4.85	41.56	4.90
V	CH_3O	i-C_3H_7O	CH_3	S	150(0.1)	1.5247	43.28	5.28	43.70	5.51
VI	C_2H_5O	i-C_3H_7O	H	S	147(0.2)	1.5295	43.28	5.28	43.48	5.51
VII	C_2H_5O	i-C_3H_7O	CH_3	S	125(0.2)	1.5272	45.14	5.68	45.41	5.82
VIII	i-C_3H_7	i-C_3H_7O	H	S	43.5–45[b]	—	47.52	5.94	47.77	6.17
IX	i-C_3H_7	i-C_3H_7O	H	O	164(0.3)	1.5028	50.17	6.27	50.10	6.67
X	i-C_3H_7	i-C_3H_7O	CH_3	S	141(1.5)	1.5382	49.21	6.31	49.02	6.40
XI	i-C_3H_7	C_2H_5O	H	S	44–46[b]	—	45.67	5.54	45.72	5.25
XII	i-C_3H_7	C_2H_5O	H	O	148(0.025)	1.5154				
XIII	CH_3	i-C_3H_7O	H	O	130(0.5)	1.5207	46.34	5.44	45.44	5.79
XIV	C_2H_5	i-C_3H_7O	H	S	140(0.5)	1.5406	45.67	5.57	46.13	6.15
XV	C_2H_5	i-C_3H_7O	H	O	150(2.5)	1.5111				
XVI	*tert*-C_4H_9	CH_3O	H	O	146(0.3)	1.5230	48.35	5.86	48.17	6.12
XVII	i-C_3H_7	i-C_3H_7	H	S	103–05[b]	—	50.17	6.32	49.88	6.62
XVIII	i-C_3H_7	i-C_3H_7	H	O	145(0.3)	1.5410				
XIX	*n*-C_3H_7	*n*-C_3H_7	H	S	83–85[b]	—	50.17	6.32	49.63	5.88

[a] All distillations carried out in a falling-film molecular still. Temperatures reported represent wall temperatures.
[b] Melting point.

Table II. Toxicity of Isopropyl Parathion and Analogs to Housefly, Honey Bee, and White Mouse



	R_1	R_2	R_3	X	Topical LD_{50}, 60°F., μg./G. Female *Musca domestica* (S_{NAIDM})	Topical LD_{50}, 60°F., μg./G. *Apis mellifera*	Selective Toxicity Ratio (LD_{50} Fly/LD_{50} Bee)	White Mouse Oral LD_{50},[a] Mg./Kg.	Anticholinesterase Activity (M, I_{50}) Fly head	Anticholinesterase Activity (M, I_{50}) Bee head	k_b, Min.$^{-1}$
I	i-C_3H_7O	i-C_3H_7O	H	S	4.7	>1000	>212	537	—	—	—
II	i-C_3H_7O	i-C_3H_7O	H	O	9.4	167	17.8	143	2.8×10^{-7}	1.0×10^{-5}	4.3×10^{-5}
III	i-C_3H_7O	i-C_3H_7O	CH_3	S	54	>1000	>18.5	>503	—	—	—
IV	CH_3O	i-C_3H_7O	H	S	1.6	1.8	1.1	21	—	—	—
V	CH_3O	i-C_3H_7O	CH_3	S	5.3	20	3.8	30–60	—	—	—
VI	C_2H_5O	i-C_3H_7O	H	S	1.2	3.0	2.5	54–67	—	—	—
VII	C_2H_5O	i-C_3H_7O	CH_3	S	8.7	52.5	6.0	82–185	—	—	—
VIII	i-C_3H_7	i-C_3H_7O	H	S	59.0	>1000	>17.0	>252	—	—	—
IX	i-C_3H_7	i-C_3H_7O	H	O	145	303	2.1	300–400	2.9×10^{-5}	1.5×10^{-3}	2.9×10^{-5}
X	i-C_3H_7	i-C_3H_7O	CH_3	S	300	>1000	3.3	—	—	—	—
XI	i-C_3H_7	C_2H_5O	H	S	25.3	>1000	>39.5	100–50	—	—	—
XII	i-C_3H_7	C_2H_5O	H	O	30.0	11.5	0.38	21–9	1.3×10^{-6}	4.5×10^{-6}	1.2×10^{-4}
XIII	CH_3	i-C_3H_7O	H	O	1.6	0.5	0.31	5–7	6.4×10^{-8}	1.6×10^{-7}	2.5×10^{-3}
XIV	C_2H_5	i-C_3H_7O	H	S	1.5	0.8	0.53	8–29	—	—	—
XV	C_2H_5	i-C_3H_7O	H	O	1.6	0.9	0.56	5–6	2.1×10^{-8}	1.5×10^{-7}	5.5×10^{-4}
XVI	$(CH_3)_3C$	CH_3O	H	O	5000	460	0.09	—	$>1.9 \times 10^{-4}$	$>1.9 \times 10^{-4}$	—
XVII	i-C_3H_7	i-C_3H_7	H	S	>5000	>1000	—	>260	—	—	—
XVIII	i-C_3H_7	i-C_3H_7	H	O	58	20	0.34	72–108	6.8×10^{-7}	2.8×10^{-6}	1.1×10^{-3}
XIX	*n*-C_3H_7	*n*-C_3H_7	H	S	4.0	3.0	0.75	56–82	—	—	—
XX	CH_3O	CH_3O	H	S	1.2	0.84	0.7	23	—	—	—
XXI	C_2H_5O	C_2H_5O	H	S	0.9	3.5	3.9	—	—	—	—
XXII	CH_3O	CH_3O	H	O	1.5	1.0	0.7	21	1.6×10^{-7}	1.0×10^{-7}	—
XXIII	C_2H_5O	C_2H_5O	H	O	1.2	0.6	0.5	—	2.7×10^{-8}	6.2×10^{-8}	

[a] Single numbers given for LD_{50} to white mouse are approximate values.

ute for fly head (20 heads per ml.) and 0.49 μ*M* AChBr hydrolyzed per minute for bee head cholinesterase (23.6 heads per ml.).

First-order rate constants (k_b) for hydrolysis of the phosphorus esters were determined at 30° C. in 0.1*M* phosphate buffer, pH 10.0, according to described procedures (Fukuto and Metcalf, 1959).

RESULTS AND DISCUSSION

Toxicity to Insects and the White Mouse. Toxicity of isopropyl parathion and its analogs to the housefly (S_{NAIDM} strain), honey bee, and white mouse is given in Table II. Several points of interest emerge from the data. Reaffirming previous observations (Dauterman and O'Brien, 1964; Metcalf and Frederickson, 1965; Metcalf and March, 1949), isopropyl parathion (I), although toxic to the housefly, is virtually nontoxic to the honey bee. The value of >1000 μg. per gram for the LD_{50} of isopropyl parathion agrees with other previous results from this laboratory but differs with the value of 522 μg. per gram reported by Dauterman and O'Brien (1964). This discrepancy in the toxicity of isopropyl parathion to the honey bee is probably due to the effect of differences in the holding temperature after dosage. The toxicity of isopropyl paraoxon (II) to the bee (LD_{50} 167 μg. per gram) is intermediate to values reported by Metcalf and Frederickson (1965) (LD_{50} 5.0 μg. per gram) and Dauterman and O'Brien (1964) (400 μg. per gram). This is a key point, since isopropyl paraoxon presumably is the metabolic intermediate responsible for the toxicity of isopropyl parathion and the value 167 μg. per gram was obtained after a number of replications. The data show that the selective toxicity ratio (*STR*)—i.e., LD_{50} honey bee/LD_{50} housefly—is greater than 212 for isopropyl parathion, while the ratio for the phosphate analog is approximately 18. For comparison, toxicity and anticholinesterase data for methyl parathion (XX), parathion (XXI), methyl paraoxon (XXII), and paraoxon (XXIII) also are given. Evidently, these compounds are equally toxic to houseflies and honey bees. Compound III, the diisopropyl analog of Sumithion (*O,O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothioate), also was nontoxic to bees and considerably less toxic to houseflies compared to I. Like Sumithion (Hollingworth *et al.*, 1967), III was nontoxic to the white mouse.

The mixed phosphate esters (IV to VII), in which one of the diisopropyl groups is substituted with methyl or ethyl, were substantially more toxic to the honey bee than isopropyl parathion, giving rise to selective toxicity ratios ranging from 1.1 to 6.0. In the case of the phosphorothionate analogs (IV and VI), safety to the honey bee is completely lost by substitution with methyl or ethyl. The thionate esters of isopropylphosphonic acid in which the isopropyl moiety is directly bonded to phosphorus (VIII, X, and XI) also were nontoxic to bees, but showed reduced toxicity to houseflies. Compound XI with moderate toxicity to houseflies and a *STR* value of >39.5 is of considerable interest because of the relatively high toxicity of its P=O analog (XII) to the honey bee. Since XII, on metabolic grounds, is the intermediate responsible for the toxicity of XI, the lack

of toxicity of the latter to the honey bee suggests that XII is not formed in the bee after treatment with XI. Similar arguments also apply for the diisopropylphosphinate esters (XVII and XVIII), although in this case the diisopropylphosphinothioate ester (XVII) is nontoxic to both the housefly and honey bee. The moderate toxicity of the phosphinate analog (XVIII) suggests that metabolic conversion of P=S to P=O does not take place in either the housefly or honey bee. In comparison, the di-*n*-propylphosphinothioate (XIX) was highly toxic to both insects. The esters of methyl- and ethylphosphonic acid (XIII to XV) were all extremely toxic to both houseflies and honey bees and in spite of the presence of the isopropyl moiety were slightly more toxic to the bee, resulting in *STR* values less than 1.

A few points concerning the mouse toxicity data in Table II also deserve comment. Isopropyl parathion is surprisingly nontoxic to the white mouse, with an LD_{50} value of 537. The 3-methyl analog (III, isopropyl Sumithion) also was nontoxic, showing no mortality at the highest dosage tested (503 mg. per kg.). In comparison, the mixed phosphate esters (IV to VII) were substantially more toxic to mice. Particularly astonishing is the high toxicity of V, *O*-methyl *O*-isopropyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate, since both the dimethyl ester (Sumithion) [LD_{50} 1250 mg. per kg.] (Hollingworth *et al.*, 1967), and the diisopropyl ester (III) are relatively nontoxic. Among the phosphonothioates, branching in the alkyl groups directly bonded to phosphorus (VIII and XI) gave compounds with less toxicity to mice compared to the corresponding case where branching is present in the alkoxy moiety (XIV). Generally speaking, the thioate esters were less toxic to mice compared to their respective oxygen analog.

Effect of Temperature on Toxicity to Honey Bee. The effect of temperature on the toxicity of isopropyl parathion and a few of its analogs is given in Table III.

The data obviously show that toxicity to the honey bee varies markedly with temperature. Isopropyl parathion (I) was greater than 2.6-fold more toxic to bees at 26.7° C. (80° F.) than at 15.6° C. (60° F.), and its phosphate analog II was twofold more toxic at the higher temperature. The most pronounced difference in toxicity is seen in the phosphonothionate (XI), which was greater than 13-fold more toxic at 26.7° C.

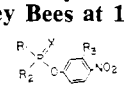
The greater toxicity of I and XI at 26.7° C. compared to 15.6° C. is probably due to two factors: increased rate of in vivo formation of the phosphate from the thionate, and increased rate of in vivo cholinesterase inhibition at the higher temperature. Bees are biologically more active at 26.7° C. than at 15.6° C. and the metabolic conversion of P=S to P=O undoubtedly takes place at a substantially faster rate at the higher temperature. Support for this is found in an investigation (Georghiou and Atkins, 1964) which showed that the toxicity of carbamate esters to the honey bee decreases with increasing temperatures—e.g., carbaryl is 3.8-fold more toxic to bees at 15.6° C. than at 26.7° C. In the case of carbamates, increase in rate of metabolism leads to faster detoxication, hence decreased toxicity (Metcalf *et al.*, 1966), while in phosphorothionates increased metabolism leads to faster rates of activation and higher toxicity. Numerous examples demonstrate increased rates of in vitro cholinesterase inhibition by organophosphorus esters with increase in temperature.

The twofold higher toxicity of isopropyl paraoxon (II) to the bee at 26.7° C. compared to 15.6° C. suggests that the rates of detoxication of phosphate esters, via hydrolysis, oxidation, or whatever do not increase as much with temperature as the rates of cholinesterase inhibition. The significantly larger difference in toxicity of isopropyl parathion at the two temperatures also suggests that the rate of P=S to P=O conversion increases faster with temperature than rates of reactions which lead to detoxication. The 14-fold difference in toxicity of XI at 15.6° and 26.7° C. and the high toxicity of the oxygen analog XII (see Table II) to the bee at 15.6° C. suggest that XI is not readily converted to XII at 15.6° C. but is converted efficiently at the higher temperatures.

These observations concerning temperature effects on bee toxicity readily account for the variance in toxicity data reported by Dauterman and O'Brien (1964) and Metcalf and Frederickson (1965). Although Dauterman and O'Brien failed to report the temperature at which the bees were held after treatment, their LD_{50} value of 522 μg. per gram for isopropyl parathion would indicate a temperature closer to 26.7° C. than 15.6° C. Finally, from a practical standpoint Table III suggests that isopropyl parathion and related compounds should be applied to areas frequented by bees on relatively cool days and carbamates should be applied on days with moderately high temperatures.

Anticholinesterase Activity. Since the toxic action of P=S esters is exerted through the P=O metabolic intermediate, the anticholinesterase activity of the P=O analogs was determined and the data are given in Table II as I_{50} values for fly head and bee head cholinesterase. With the exception of XVI, all compounds are more active in inhibiting fly head than bee head cholinesterase. XVI was inactive against the enzyme from either source at the highest concentration tested. Of the compounds examined, II and IX were substantially more active in inhibiting fly cholinesterase compared to bee, with II showing 36-fold and IX 50-fold greater inhibitory activity against the enzyme from the fly. The I_{50} values reported for II are in good agreement with results previously reported (Dauterman and O'Brien, 1964). In contrast to II and IX, the difference in anticholinesterase activity between the two enzymes is markedly less for

Table III. Relative Toxicity of Isopropyl Parathion and Analogs to Honey Bees at 15.6° and 26.7° C.



	R_1	R_2	R_3	X	LD_{50}, μg./G. 15.6°C.	26.7°C.
I	i-C_3H_7O	i-C_3H_7O	H	S	>1000	370
II	i-C_3H_7O	i-C_3H_7O	H	O	167	82
III	i-C_3H_7O	i-C_3H_7O	CH_3	S	>1000	>1000
XI	i-C_3H_7	C_2H_5O	H	S	>1000	72

compounds XII, XIII, XV, and XVIII, giving values ranging from 2.4 to 7.0 greater inhibitory activity against housefly cholinesterase compared to honey bee. This reduced difference in anticholinesterase appears to have a profound effect on selective toxicity, as discussed below.

To account for the difference in anticholinesterase activity of these compounds against cholinesterase from the housefly and honey bee, isopropyl paraoxon (II) was examined in greater detail by the kinetic analysis developed by Main (1964). The equilibrium constant for reversible dissociation of the enzyme-inhibitor complex to enzyme and inhibitor (K_i) and the rate constant for the phosphorylation reaction (k_p) as depicted below were determined for II. E and I are the enzyme and inhibitor, EI is the enzyme-inhibitor complex, and EI' is the phosphorylated enzyme. The bimolecular inhibition constant, k_e, is equal to k_p/K_i.

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} EI \xrightarrow{k_p} EI' + p$$

$$EI \xrightarrow{k_e} EI' + p$$

The usual plot of $1/[I]$ against $\Delta t/\Delta \ln V$ gave good straight lines, as illustrated in Figure 1 for the inhibition of honey bee cholinesterase by II. The slope and intercept were determined by least square regression analysis and Figure 1 shows that significant intercepts, hence significant values of K_i and k_p, were obtained. The values for K_i (M), k_p (min.$^{-1}$), and k_e (M^{-1} min.$^{-1}$) for isopropyl paraoxon are: for honey bee cholinesterase K_i 9.1 $\times$ 10^{-4}, k_p 2.2, and k_e 2.4 $\times$ 10^3; for housefly cholinesterase K_i 9.5 $\times$ 10^{-5}, k_p 8.3, and k_e 8.8 $\times$ 10^4. These values are in reasonable agreement with those reported by Main and Iverson (1966) for the inhibition of human erythrocyte cholinesterase by diisopropyl phosphorofluoridate. k_e for housefly cholinesterase is approximately 37-fold larger than for honey bee cholinesterase, in excellent agreement with the I_{50} values given in Table I. Examination of K_i and k_p values shows that this large difference in anticholinesterase activity is caused by the 9.6-fold larger K_i value for honey bee compared to housefly and the 3.9-fold larger k_p value for housefly enzyme. Thus, according to these constants, isopropyl paraoxon combines more readily with fly head cholinesterase to form the enzyme-inhibitor complex and also phosphorylates the fly enzymes at a faster rate than honey bee cholinesterase.

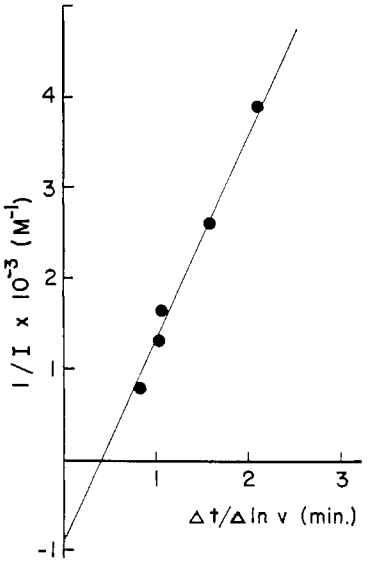


Figure 1. Plot of reciprocal of inhibition rate against reciprocal of inhibitor concentration to obtain k_e, K_i, and k_p for isopropyl paraoxon with honey bee head ChE

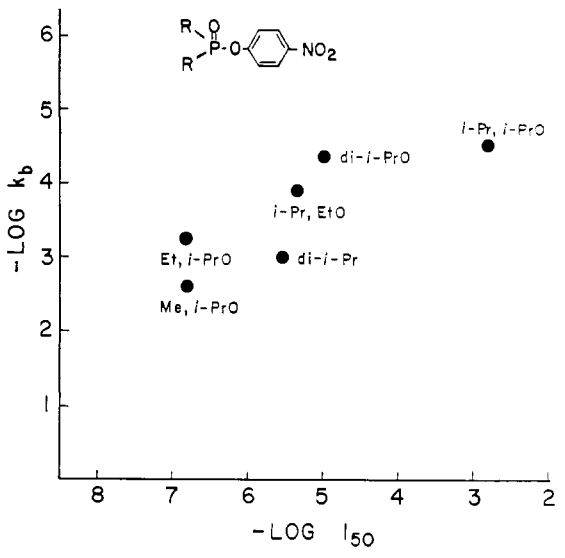


Figure 2. Plot of negative log of honey bee ChE inhibition I_{50} value against negative log of k_b of isopropyl paraoxon and several of its phosphonate and phosphinate analogs

The last column in Table II gives pseudo-first-order hydrolysis constants of the various P=O compounds in pH 10 phosphate buffer. Examination of the data, which include hydrolysis constants and anticholinesterase values for a phosphate, a phosphinate, and several phosphonates, shows that in general anticholinesterase activity is directly related to the reactivity of the molecule as estimated by its susceptibility to alkaline hydrolysis. The relationship between the logarithms of the I_{50} values and hydrolysis constants for honey bee head cholinesterase is presented graphically in Figure 2. A similar plot (not given here) may be obtained with housefly cholinesterase. Attempt was made to correlate anticholinesterase activity with Taft's σ^* and E_s constants (Hansch and Deutsch, 1966), but there were too few compounds to obtain significant correlation.

Cholinesterase Inhibition and Toxicity. Examination of Table II reveals a general absence of correlation between the toxicity of the compounds and relative anticholinesterase activity of the P=O esters. For example, compounds XII, XIII, XV, and XVIII with selective inhibition ratios (I_{50} honey bee ChE/I_{50} fly head ChE) ranging from 2.4 to 7.0 are actually more toxic to honey bees on a weight basis, with selective toxicity ratios ranging from approximately 0.3 to 0.6.

Compound IX with a selective inhibition ratio of 50 is only twofold more toxic to the fly than bee. The closest agreement between anticholinesterase activity and insecticidal toxicity was found with isopropyl paraoxon (II) with selective inhibition and toxicity ratios of 36 and 18, respectively. Therefore, it appears with isopropyl paraoxon that selectivity is in part due to the difference in susceptibility of the cholinesterases to inhibition. These values agree favorably with the results of Dauterman and O'Brien (1965), who found that isopropyl paraoxon was 22 times more toxic to flies than bees and 40 times more active against fly cholinesterase than bee cholinesterase.

In spite of the reasonable correlation between toxicity and anticholinesterase activity found with isopropyl paraoxon, the unusually large selective toxicity ratio (>212) for isopropyl parathion dictates that other factors in addition to selectivity in target enzyme inhibition must be considered. This point is amplified further if we examine the data for *O*-ethyl *p*-nitrophenyl isopropylphosphonothioate (XI) and the analogous phosphonate (XII). Table II shows that the phosphonate (XII) inactivates housefly cholinesterase about 3.5 times faster than bee cholinesterase but is 2.6 times more toxic to bees than flies. On the other hand, the phosphonothioate (XI) is essentially nontoxic to bees while showing moderate toxicity to flies, resulting in a selective toxicity ratio of >39.5. The much greater toxicity of XII compared to XI to bees suggests that in vivo P=S to P=O conversion of XI to XII (the ultimate toxicant) does not take place in the bee or at such a slow rate that the level of XII never reaches a toxic level owing to concurrent detoxication reactions. Similar arguments also apply for the selectivity of isopropyl parathion. Another paper (Camp *et al.*, 1969) reports on the comparative metabolism of isopropyl parathion in the housefly and honey bee, in an attempt to verify this point.

NOMENCLATURE

- E = enzyme
- EI = enzyme-inhibitor complex
- EI′ = phosphorylated enzyme
- K_i = equilibrium constant for reversible dissociation of EI to E and I, *M*
- k_p = rate constant for phosphorylation, $min.^{-1}$
- k_e = bimolecular inhibition constant, M^{-1} $min.^{-1}$
- k_b = first-order hydrolysis constant, $min.^{-1}$
- *STR* = selective toxicity ratio, LD_{50} honey bee/LD_{50} housefly
- σ^* = Taft's polar substituent constant
- E_s = Taft's steric substituent constant

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