Structure, Reactivity, and Biological Activity of *O*-(Diethyl Phosphoryl)Oximes and *O*-(Methylcarbamoyl)Oximes of Substituted Acetophenones and α-Substituted Benzaldehydes

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A series of *O*-(diethyl phosphoryl) and *O*-(methylcarbamoyl)oximes of substituted acetophenones and α-substituted benzaldehydes were evaluated for anticholinesterase activity, toxicity to insects, and for chemical reactivity. Multiple regression analysis was used to correlate reactivity and biological data with free energy parameters. Excellent correlation was obtained between electronic effects, reactivity and anticholinesterase activity for the ring-substituted acetophenone *O*-(diethyl phosphoryl)oximes and *O*-(methylcarbamoyl)oximes. With the α-substituted benzaldehyde *O*-(methylcarbamoyl)oximes, the addition of Hansch's π constant to the regression equation was necessary to provide correlation with anticholinesterase activity. An unusual example of the Beckmann rearrangement was discovered and the high reactivity of substituted acetophenone *O*-(diethyl phosphoryl)oximes containing electron-donating substituents is explained in terms of this rearrangement.

Since the report by Hackley *et al.* (1959) describing the potent anticholinesterase activity and toxicity of *O*-(isopropyl methylphosphoryl)-4-formyl-1-methylpyridinium iodide oxime, a phosphonate ester of an oxime, a number of carbamate and phosphate esters of aldoximes and ketoximes have been developed as promising new insecticides. Outstanding among these compounds are Temik [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, Union Carbide], Tranid [*exo*-3-chloro-*endo*-6-cyano-2-norbornanone *O*-methylcarbamoyl)oxime, Union Carbide], Lannate [methyl-*O*-(methylcarbamoyl)-thiolacetohydroxamate, Du Pont], Bayer 77488 (*O,O*-diethyl thiophosphoryl *O*-α-cyanobenzaldoxime), and 2-oximino-1,3-dithiolane methylcarbamate (Kilsheimer and Manning, 1962; Payne *et al.*, 1967; Weiden, 1968; Addor, 1965). Examination of the structures of these compounds reveals wide variation in the make-up of the oxime moiety and, indeed, the variety of structural modifications which can be made in the oxime portion of the molecule and the outstanding insecticidal activity of some of these compounds have stimulated unusual interest in carbamoyl- and phosphoryl-oximes.

In previous reports from this laboratory (Fukuto and Metcalf, 1956; Metcalf and Fukuto, 1965; Metcalf and Fukuto, 1967; Fukuto *et al.*, 1967; Fukuto, 1969), the authors have attempted to correlate biological activity with chemical structure and physical organic parameters of a variety of substituted phenyl methylcarbamates and diethyl phosphates. This paper represents a continuation of our efforts in this area and is concerned with the relationship between chemical structure, biological activity, and reactivity of a series of *O*-(methylcarbamoyl)- and *O*-(diethyl phosphoryl)oximes of ring-substituted acetophenones and α-substituted benzaldehydes. Multiple regression analysis was used to correlate biological activity and chemical reactivity with different free energy parameters for the various substituents attached to the benzene ring and to the α-carbon atoms.

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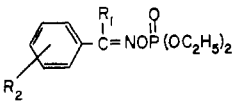
MATERIALS AND METHODS

Substituted acetophenones. *o*-, *m*-, *p*-Methylacetophenone, *o*-, *m*-, *p*-methoxyacetophenone, *m*-, *p*-nitroacetophenone, *p*-chloroacetophenone, *p*-bromoacetophenone, propiophenone, butyrophenone, isobutyrophenone, and valerophenone were obtained from the Aldrich Chemical Co. *p*-Cyanoacetophenone, *p*-fluoroacetophenone, *o*-, *m*-, and *p*-trifluoromethyl acetophenone were obtained from K and K Laboratories. *o*-Nitroacetophenone was prepared according to Walker and Hauser (1948), *p*-iodoacetophenone according to Matsui (1942), 2,2,2-trifluoroacetophenone according to Dishart and Levine (1956), and 2-dimethylaminoacetophenone according to Letsinger and Collat (1952).

Oximes. Oximes of the above acetophenones were prepared in the conventional manner by allowing a mixture of hydroxylamine hydrochloride (1.3 eq.), acetophenone and sodium hydroxide (1.3 eq.) to stand in aqueous ethyl alcohol for 24 hours, then heating the mixture at reflux for 2 hours. The oximes were isolated as crystalline solids and purified by repeated crystallizations from *n*-hexane, benzene, or mixtures of *n*-hexane and benzene. Attempt was not made to separate *syn* and *anti* isomers but in most cases evidently a single isomer with sharp melting point was obtained. Melting points (°C.) of the oximes prepared as described above are given as follows for the various substituted acetophenones and related ketones: *o*-methyl 59 to 61, *m*-methyl 54 to 6, *p*-methyl 86 to 8, *o*-methoxy 94 to 5, *m*-methoxy, b.p. 76 to 80 (0.1 mm), *p*-methoxy 84 to 6, *o*-trifluoromethyl 105 to 12, *m*-trifluoromethyl 78 to 82, *p*-trifluoromethyl 102 to 6, *o*-nitro 115 to 7, *m*-nitro 131 to 2, *p*-nitro 172 to 4, *p*-Cl 93 to 5, *p*-bromo 127 to 9, *p*-iodo 159 to 61, *p*-fluoro 74 to 5, *p*-cyano 158 to 60, propiophenone 53 to 4, butyrophenone 48 to 50, isobutyrophenone 88 to 90, valerophenone 50 to 2, benzophenone 141 to 3, and 2,2,2-trifluoroacetophenone 69 to 71.5. α-Cyanobenzaldoxime, m.p. 124 to 5°C., was prepared by nitrosylation of phenylacetonitrile (Perrot, 1934). α-Methylthiobenzaldoxime, m.p. 82 to 3°C., was prepared according to Benn (1964).

***O*-(Diethyl Phosphoryl)Oximes.** All *O*-(diethyl phosphoryl)oximes were prepared by reacting the sodium salt of

Table I. Physical and Biological Properties of Substituted Acetophenone and Benzaldehyde *O*-(Diethyl Phosphoryl)Oxime



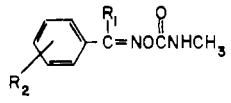
	R_1	R_2	Elemental Analysis Calcd.	Found	Distillation[a] Temp., °C. (0.025 Mm.)	n_D^{25}	$I_{50}(M)$ Fly ChE	House Fly, LD_{50} (μg./g.)	*Culex*, LC_{50} (P.p.m.)	k_b
1	CH_3	H	C 53.64	53.37	120–4	1.4921	2.3×10^{-8}	>500	1.25	6.26×10^{-3}
			H 6.65	6.84						
2	CH_3	*o*-CH_3	C 54.72	54.23	135	1.4996	1.2×10^{-7}	30	1.4	—
			H 7.06	7.12						
3	CH_3	*m*-CH_3	C 54.72	54.95	130	1.5108	5.1×10^{-5}	>500	>10	—
			H 7.06	6.85						
4	CH_3	*p*-CH_3	C 54.72	54.86	100	1.5197	1.1×10^{-7}	180	0.59	4.2×10^{-2}
			H 7.06	6.92						
5	CH_3	*o*-OCH_3	C 51.82	52.07	118	1.5068	9.4×10^{-8}	>500	>10	—
			H 6.69	6.89						
6	CH_3	*m*-OCH_3	C 51.82	51.91	160	1.5116	7.0×10^{-7}	>500	>10	7.4×10^{-3}
			H 6.69	6.80						
7	CH_3	*p*-OCH_3	C 51.82	51.27	98	1.4993	7.2×10^{-9}	27	1.1	6.0×10^{-1}
			H 6.69	6.37						
8	CH_3	*o*-CF_3	C 46.02	46.64	95	—	9.0×10^{-4}	>500	>10	—
			H 5.04	5.29						
9	CH_3	*m*-CF_3	C 46.02	46.95	100	1.4713	1.7×10^{-4}	>500	>10	—
			H 5.04	5.43						
10	CH_3	*p*-CF_3	C 46.02	45.92	108	1.4708	4.2×10^{-6}	>500	>10	—
			H 5.04	6.10						
11	CH_3	*o*-NO_2	C 45.57	45.19	135	1.5082	9.8×10^{-8}	400	>10	—
			H 5.41	5.86						
12	CH_3	*m*-NO_2	C 45.57	45.06	m.p. 41–3	—	8.0×10^{-6}	>500	>10	3.7×10^{-3}
			H 5.41	5.42						
13	CH_3	*p*-NO_2	C 45.57	46.31	150	1.5267	6.7×10^{-7}	370	>10	4.2×10^{-3}
			H 5.41	5.93						
14	CH_3	*p*-CN	C 52.69	52.19	m.p. 44–6	—	1.9×10^{-5}	>500	>10	—
			H 5.79	6.00						
15	CH_3	*p*-F	C 49.83	50.04	105	1.4958	2.9×10^{-6}	>500	>10	—
			H 5.92	6.05						
16	CH_3	*p*-Cl	C 47.14	47.22	100	1.5205	2.0×10^{-6}	>500	>10	3.4×10^{-3}
			H 5.63	5.66						
17	CH_3	*p*-Br	C 41.14	41.23	100	1.5349	8.2×10^{-7}	>500	>10	2.9×10^{-3}
			H 4.91	4.90						
18	CH_3	*p*-I	C 36.28	36.03	100	1.5596	3.4×10^{-7}	>500	>10	2.4×10^{-3}
			H 4.33	4.20						
19	C_2H_5	H	C 54.72	54.90	106	1.5077	9.4×10^{-4}	>500	>10	1.3×10^{-3}
			H 7.05	7.07						
20	*i*-C_3H_7	H	C 56.17	56.19	100	1.4945	2.3×10^{-8}	20	1.0	8.7×10^{-5}
			H 7.40	7.66						
21	*n*-C_4H_9	H	C 57.31	57.65	93	1.5081	$>1 \times 10^{-3}$	>500	>10	—
			H 7.69	8.22						
22	SCH_3	H	C 47.52	48.08	128	1.5296	2.6×10^{-5}	>500	>10	9.58×10^{-3}
			H 5.98	6.42						
23	CN	H	C 51.06	51.20	150	1.5051	1.6×10^{-9}	2.25	0.02	—
			H 5.35	5.12						
24	CF_3	H	C 44.31	44.71	107–8	1.4594	3.7×10^{-8}	27.5	6	—
			H 4.65	4.28	(0.005 Mm.)					
25	C_6H_5	H	C 61.26	61.38	140	1.5499	5.6×10^{-7}	>500	>10	—
			H 6.05	6.07						

[a] Except for **24**, all compounds were distilled in a falling-film molecular still.

the oxime with diethyl phosphorochloridate in toluene. The following procedure for the synthesis of propiophenone *O*-(diethyl phosphoryl)oxime, compound **19** in Table I, is typical. To a mixture of 11.4 grams of dried propiophenone oxime sodium salt and 75 ml. of anhydrous toluene was added dropwise 8.0 grams diethyl phosphorochloridate. The mixture was warmed gently for 1 hour, cooled, washed three times with 5% sodium hydroxide, twice with water, and dried over anhydrous sodium sulfate. Distillation through a falling-film molecular still gave a colorless, viscous liquid, collected at a wall temperature of 106 to 108°C. (0.025 mm.). Physical properties and elemental analysis of all phosphate esters are given in Table I. Microanalyses were by C. F. Geiger, Ontario, Calif.

***O*-(Methylcarbamoyl)Oximes.** The *O*-(methylcarbamoyl)-oximes were prepared in the usual manner by reacting the oxime with methylisocyanate (Kolbezen *et al.*, 1954). Triethylamine was used as a catalyst in those cases where reaction did not take place spontaneously. Crystalline carbamates were purified by recrystallization from hexane, benzene, or mixtures of hexane-benzene. Liquid carbamates were purified by distillation or by preparative thin-layer chromatography. Elemental analyses and physical properties are given in Table II. Only in a single case, compounds **46** and **47**, did

Table II. Physical and Biological Properties of Substituted Acetophenone and Benzaldehyde *O*-(Methylcarbamoyl)Oximes



	R_1	R_2	Elemental Analysis Calcd.	Elemental Analysis Found	M.P., °C.	$I_{50}(M)$ Fly ChE	House Fly LD_{50} (μg./g.) Alone	House Fly LD_{50} (μg./g.) 1:5 P.B.	*Culex* lc_{50} (P.p.m.)	k_b
26	CH_3	H	C 62.48 H 6.29	62.53 6.40	93–7	7.1×10^{-5}	>500	200	>10	4.9×10^{-3}
27	CH_3	*m*-CH_3	C 64.08 H 6.84	64.25 6.66	123–6	3.8×10^{-5}	>500	75	—	—
28	CH_3	*p*-CH_3	C 64.08 H 6.84	64.26 6.48	100–4	9.0×10^{-5}	>500	450	>10	4.65×10^{-3}
29	CH_3	*o*-CH_3	C 59.45 H 6.35	60.29 6.44		1.5×10^{-4}	>500	105	>10	—
30	CH_3	*m*-OCH_3	C 59.45 H 6.35	58.92 6.12	92–5	1.5×10^{-4}	>500	135	>10	8.15×10^{-3}
31	CH_3	*p*-OCH_3	C 59.45 H 6.35	60.24 6.13	142–3	1.9×10^{-4}	>500	>500	>10	—
32	CH_3	*o*-CF_3	C 50.77 H 4.26	51.55 4.07	130 (0.05 Mm.)	3.7×10^{-5}	>500	38	>10	—
33	CH_3	*m*-CF_3	C 50.77 H 4.26	50.91 4.15	90–3	1.1×10^{-5}	>500	17.5	>10	—
34	CH_3	*p*-CF_3	C 50.77 H 4.26	50.89 4.34	138–40	8.0×10^{-5}	>500	>500	>10	—
35	CH_3	*o*-NO_2	C 50.63 H 4.67	50.91 4.68	98–101	1.0×10^{-5}	>500	36.5	2.7	—
36	CH_3	*m*-NO_2	C 50.63 H 4.67	51.00 4.65	165–8	4.4×10^{-6}	>500	>500	>10	1.46×10^{-2}
37	CH_3	*p*-NO_2	C 50.63 H 4.67	50.77 4.40	188–90	8.0×10^{-6}	>500	>500	>10	1.45×10^{-2}
38	CH_3	*p*-CN	C 60.82 H 5.10	61.32 4.99	146–8	1.7×10^{-5}	>500	80	>10	—
39	CH_3	*p*-F	C 57.14 H 5.28	57.71 5.12	129–30	1.1×10^{-4}	>500	67.5	>10	—
40	CH_3	*p*-Cl	C 52.99 H 4.89	53.55 4.81	131–2	3.5×10^{-5}	>500	360	>10	9.7×10^{-3}
41	CH_3	*p*-Br	C 44.29 H 4.09	44.44 4.17	130–4	3.9×10^{-5}	>500	>500	>10	1.1×10^{-2}
42	CH_3	*p*-I	C 37.76 H 3.48	38.41 3.54	132–3	3.5×10^{-5}	>500	>500	>10	1.1×10^{-2}
43	H	H	C 60.66 H 6.04	60.60 6.02	94–6	9.2×10^{-5}	500	55	>10	—
44	C_2H_5	H	C 64.06 H 6.84	64.69 6.37	81–3	2.8×10^{-5}	500	27	6.2	—
45	*n*-C_3H_7	H	C 65.43 H 7.32	65.88 7.89	128 (0.5 Mm.)	1.3×10^{-4}	>500	60	>10	—
46	*i*-C_3H_7	H	C 65.43 H 7.32	65.12 7.37	51–6	1.0×10^{-5}	>500	33	2.8	9.8×10^{-4}
47	*i*-C_3H_7	H	C 65.43 H 7.32	66.29 6.90	101–3	5.7×10^{-6}	>500	18	2.4	—
48	SCH_3	H	C 53.55 H 5.40	53.95 5.50	112–4	7.8×10^{-6}	>500	8.8	3.0	—
49	CN	H	C 59.10 H 4.46	59.84 4.72	167–79	1.4×10^{-4}	>500	245	>10	—
50	CF_3	H	C 48.78 H 3.68	49.29 3.86	77–8	5.8×10^{-7}	30	9.5	0.6	—
51	CH_2NMe_2	H H	C 61.24 7.28	61.56 7.12	85–6	1.5×10^{-5}	>500	185	>10	—

fractional crystallization produce the two geometric isomers of the carbamoyloxime.

The molar concentration for 50% inhibition (I_{50}) was determined for house fly head cholinesterase as described previously (Fukuto and Metcalf, 1956). Techniques for determination of insecticidal activity against susceptible female house flies (*Musca domestica* L., Snaidm strain) and mosquito larvae (*Culex pipiens quinquefasciatus* Say.) have been described (Mulla *et al.*, 1966; March *et al.*, 1964).

Hydrolysis constants (k_b) at 30°C. of the various *O*-(methylcarbamoyl)oximes and *O*-(diethyl phosphoryl)oximes in 0.01*M* sodium hydroxide were determined spectrophotometrically by estimating the amount of product remaining after measured time intervals in a Unicam SP-800 spectrophotometer at 250 to 285 mμ. Spectra of the oxime esters and hydrolysis products were determined prior to kinetic studies to show that overlaps in pertinent absorption peaks were not present. Hydrolysis constants of a number of compounds listed in Tables I and II could not be determined owing to overlap in absorption peaks. The experimental procedure for the determination and calculation of the pseudo first-order rate constants has been reported previously (Fukuto *et al.*, 1967).

Identification of the products from the alkaline hydrolysis of

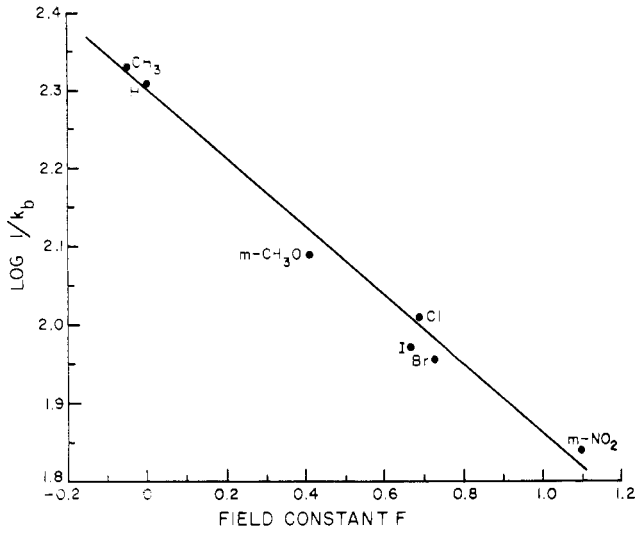


Figure 1. Relation between alkaline hydrolysis constant k_b and field constant F for *N*-methylcarbamates of *m*- and *p*-substituted acetophenone oximes

p-nitro-, *p*-methoxy-, and *p*-methylacetophenone *O*-(diethyl phosphoryl)oximes was carried out as follows. To 100 ml. of 0.01*M* aqueous sodium hydroxide was added 0.1 gram of *p*-nitroacetophenone *O*-(diethyl phosphoryl)oxime dissolved in 1 ml. of ethyl alcohol. After continuous shaking for 24 hours, the mixture was washed with ether, neutralized with concentrated hydrochloric acid, and the product was extracted with ether. Drying over sodium sulfate and evaporation of the solvent gave *p*-nitroacetophenone oxime as the sole product, a yellow crystalline solid, m.p. 173 to 4°C., literature 174°C. (Heilbron, 1965). The same procedure with the *p*-methoxy analog gave only *p*-methoxyacetanilide, m.p. 128 to 9°C., literature 130 to 2°C. (Heilbron, 1965). A mixture melting with an authentic sample of *p*-methoxyacetanilide was 128 to 9°C. Hydrolysis of the *p*-methyl analog gave a product which melted at 143 to 7°C., literature m.p. for *p*-methylacetanilide 146°C. (Heilbron, 1965). Infrared spectrum (Perkin-Elmer Model 21) of the crude product gave evidence for presence of a small amount of the oxime as the side product. A Varian A-60 spectrometer was used for nmr analysis.

DISCUSSION OF RESULTS

Biological data, including anticholinesterase activity (I_{50}), toxicity to the house fly and mosquito larvae, and pseudo first-order hydrolysis constants in dilute sodium hydroxide for the *O*-(diethyl phosphoryl)oximes are given in Table I and for the *O*-(methylcarbamoyl)oximes in Table II. In the case of the *O*-(methylcarbamoyl)oximes toxicity data for the house fly with 5 to 1 parts piperonyl butoxide synergist also are given. Correlation between free energy parameters of the various substituents on the benzene ring and α-carbon atom with alkaline hydrolysis rates and biological activity was accomplished by means of multiple regression analysis.

Alkaline Hydrolysis. Rates of hydrolysis in 0.01*M* sodium hydroxide were determined to estimate the order of reactivity of the *O*-(methylcarbamoyl)oximes and *O*-(diethyl phosphoryl)oximes. The limited data in Tables I and II show that in the case of the ring-substituted *O*-(methylcarbamoyl)-oximes a direct relationship exists between the electron-withdrawing tendency of the substituent and the first-order hydrolysis constant (k_b) but the relationship is inverse with the *O*-(diethyl phosphoryl)oximes—*i.e.*, electron-withdrawing substituents result in more stable phosphates.

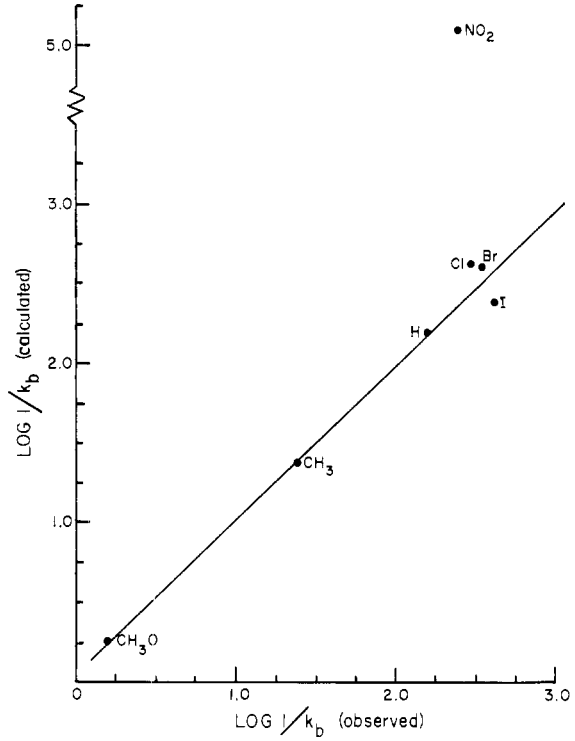


Figure 2. Correlation between observed k_b for alkaline hydrolysis and k_b calculated from F and R values according to Equation 2 for *p*-substituted acetophenone oxime diethyl phosphates

Multiple regression analysis of alkaline hydrolysis rates of the *p*-substituted acetophenone *O*-(methylcarbamoyl)oximes (Table II) and with electronic effects as estimated by field (F) and resonance (R) constants of Swain and Lupton (1968) produced the equation

$$\log 1/k_b = 2.301 - 0.44F \qquad n = 6 \qquad r = 0.993 \quad (1)$$

where n is the number of compounds and r is the correlation coefficient. Inclusion of R in the equation raised r to 0.997, an insignificant amount. The correlation of these hydrolysis rates with σ_p (Hine, 1962) was not as satisfactory ($r = 0.89$). There were too few *m*-substituted compounds to make a separate analysis. However, inclusion of the two *m*-substituted compounds (*m*-OCH_3 and *m*-NO_2) with the *p*-substituted compounds in the plot of log $1/k_b$ against F values gave the excellent linear relationship shown in Figure 1. The results imply that field effects from the *m*- and *p*-positions are the same and this is probably due to the substantial distance between the substituent and the reaction center (carbonyl carbon).

Regression analysis of F and R constants with alkaline hydrolysis constants in Table I produced the equation

$$\log 1/k_b = 2.218 + 1.862F + 5.457R \qquad n = 6 \qquad r = 0.99 \quad (2)$$

for the *p*-substituted acetophenone *O*-(diethyl phosphoryl)-oximes (exclusive of the *p*-NO_2 compound). The *p*-nitro compound **13** did not fit into the correlation as shown graphically in Figure 2. Correlation was less satisfactory with σ_p with $r = 0.93$. Thus, it appears that alkaline hydrolysis rates of the *O*-(diethyl phosphoryl)oximes is linearly correlated with the electron-donating tendency of the substituent as represented by field and resonance effects. This relationship is inverse to the one found for the substituted *O*-(methylcarbamoyl)oximes where electron-withdrawing groups favor alkaline hydrolysis.

These results are difficult to rationalize in terms of a

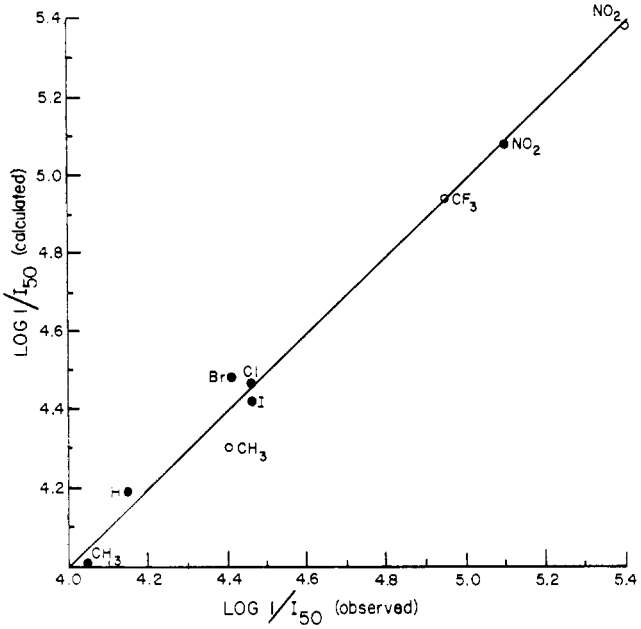


Figure 3. Correlation between observed I_{50} and I_{50} calculated from F and R values according to Equations 3 and 4 for p-substituted (●) and m-substituted (○) acetophenone oxime N-methylcarbamates

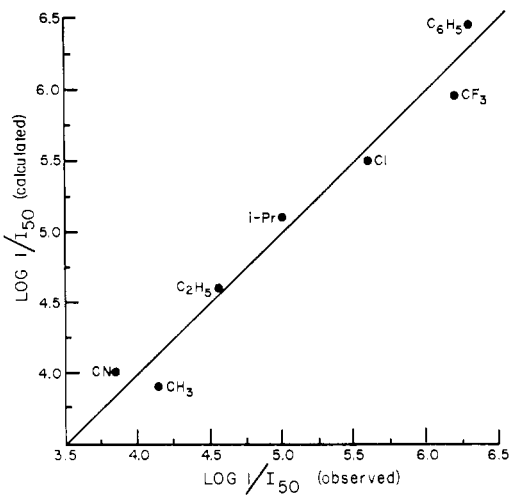
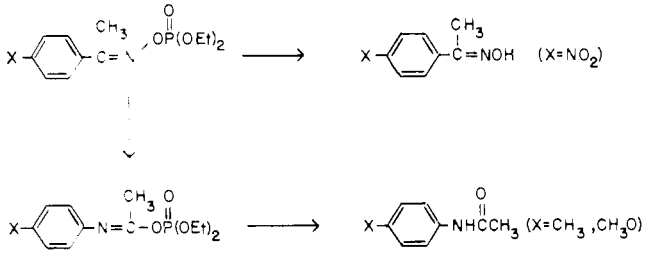


Figure 4. Correlation between observed I_{50} and I_{50} calculated from F and π values according to Equation 5 for α-substituted benzaldoxime N-methylcarbamates

mechanism involving nucleophilic attack of hydroxide ion on the phosphorus atom. In the case of substituted phenyl diethyl phosphates, electron-withdrawing substituents produced esters with less stability to alkaline hydrolysis than those with electron-donating substituents (Fukuto and Metcalf, 1956). For this reason, an analysis of the hydrolysis products was made to determine if the phosphate and carbamate esters hydrolyzed with different mechanisms. Treatment of representative O-(diethyl phosphoryl)oximes at room temperature with 0.01M sodium hydroxide for 24 hours gave the following results. p-Nitroacetophenone O-(diethyl phosphoryl)oxime produced p-nitroacetophenone oxime, the product normally expected by attack of hydroxide on phosphorus resulting in displacement of the oxime moiety. On the other hand, p-methoxy- and p-methylacetophenone O-(diethyl phosphoryl)oximes, each representing substituents on the opposite end of the σ scale respective to nitro, gave, respectively, p-methoxyacetanilide and p-methylacetanilide as the main products, presumably through the intermediate enol phosphate as indicated below.



Similar treatment of the O-(methylcarbamoyl)oximes of p-nitro- and p-methoxyacetophenone with 0.01M sodium hydroxide gave the oxime in both cases. The equation leading to the anilide obviously is a result of the Beckmann rearrangement facilitated by electron-donating substituents. This unusual example of the Beckmann rearrangement currently is under further investigation, particularly since the rearrangement may have a bearing on the anticholinesterase properties of these esters. The effect of the rearrangement on reactivity is discussed in the next section.

Cholinesterase Inhibition. Anticholinesterase data in Tables I and II show that the O-(diethyl phosphoryl)oximes are, on the whole, stronger inhibitors of house fly cholinesterase than the O-(methylcarbamoyl)oximes. Regression analysis of F, R, and π with anticholinesterase activity (Hansch and Deutsch, 1966) gave Equation 3 for the 4-substituted and Equation 4 for the 3-substituted acetophenone O-(methylcarbamoyl)oximes which best fit the data.

$$\log 1/I_{50} = 4.189 + 0.65F + 1.06R \qquad n = 6 \qquad r = 0.993 \quad (3)$$

$$\log 1/I_{50} = 4.33 + 0.952F \qquad n = 4 \qquad r = 0.97 \quad (4)$$

Inclusion of π in either Equation 3 or 4 had no significant effect on the correlation. The results are presented graphically in Figure 3.

The absence of π in Equations 3 and 4 suggests that molecular complementarity of the O-(methylcarbamoyl)oximes with the cholinesterase active site is not an important factor in the inhibition process as is the case with the substituted phenyl methylcarbamates (Metcalf and Fukuto, 1967; Hansch and Deutsch, 1966). Equations 3 and 4 which show that anticholinesterase activity is dependent only on field and resonance effects suggests that cholinesterase inhibition by the substituted acetophenone O-(methylcarbamoyl)oximes is determined by the reactivity of the carbamoyl moiety, analogous to the hydrolysis of these esters.

The anticholinesterase activity of the α-substituted benzaldehyde O-(methylcarbamoyl)oximes (compounds **43–51**) was readily accounted for by electronic and hydrophobic effects as estimated by F, R, and π. Regression analysis produced the equation below.

$$\log 1/I_{50} = 3.16 + 1.62\pi + 1.66F \qquad n = 7 \qquad r = 0.98 \quad (5)$$

The predicted $vs.$ observed values of log $1/I_{50}$ are shown graphically in Figure 4. Inclusion of R in the equation had no effect on the correlation coefficient. Equation 5 shows that hydrophobic effects (π) and polar effects (F) are equally weighted, suggesting that steric interaction between the α-substituent and anionic site of the enzyme, and the reactivity of the carbamate to produce a carbamoylated enzyme, play equally important roles in the overall inhibition process.

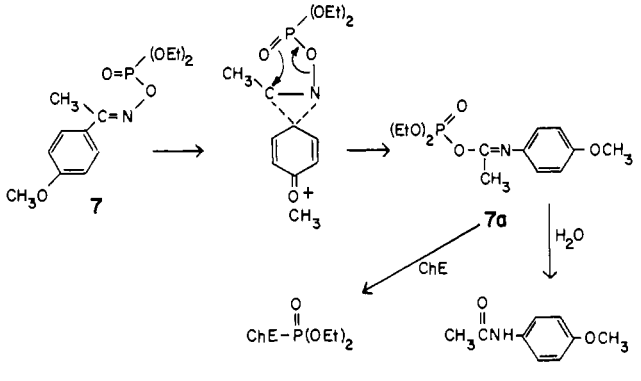
Therefore, substituents possessing large positive F and π values should produce α-substituted benzaldehyde O-(methylcarbamoyl)oximes with high anticholinesterase activity.

Observations of molecular models of the α-substituted benzaldehyde O-(methylcarbamoyl)oximes and a plaster cast of the model of acetylcholine suggest that the α-substituent can interact with the anionic site of cholinesterase when the carbonyl group is engaged at the esteratic site about 5 Å away. Thus, the increasing affinity for cholinesterase shown by **43** α-H, I_{50} 9.2×10^{-5}; **26** α-CH_3, I_{50} 7.1×10^{-5}; **44** α-C_2H_5, I_{50} 2.8×10^{-5}; and **47** α-$CH(CH_3)_2$, I_{50} 5.7×10^{-6}, can be explained by the increasing van der Waals' dispersion forces at the anionic site. A more direct proof of this interaction was obtained by the increased affinity of **51** α-$CH_2N(CH_3)_2$, I_{50} 1.5×10^{-5}, through quaternization with methyl iodide to give α-$CH_2N^+(CH_3)_3$, I_{50} 1.75×10^{-6}.

Multiple regression analysis of cholinesterase inhibition and free energy parameters with the p-substituted acetophenone O-(diethyl phosphoryl)oximes in Table II produced Equation 6 which give best fit to the data.

$$\log 1/I_{50} = 7.19 - 2.37F - 2.47R \qquad n = 9 \qquad r = 0.89 \tag{6}$$

As with the hydrolysis study, the p-nitro phosphate **13** proved to be exceptional and was not included in the analysis. The correlation represented by Equation 6 is shown graphically in Figure 5. The results show, as was found with the hydrolysis of these esters, that anticholinesterase activity is directly related to the electron-donating tendency of the substituents. Equation 6 and also the fact that O-(diethyl phosphoryl)oximes containing electron-donating substituents undergo the Beckmann rearrangement suggests the possibility that a reactive intermediate such as **7a** obtained from **7** may be responsible in part for the anticholinesterase data. The Beckmann rearrangement of oxime phosphates and sulfonates has been demonstrated by others (Kenner *et al.*, 1956).



The intermediate **7a,** the imido or enol phosphate of p-methoxyacetanilide, is a highly reactive phosphate ester and should react rapidly with both water and the cholinesterase enzyme. The formation of the anilide upon hydrolysis of **7** suggests that the compound examined for reactivity and biological activity as **7** is actually **7a,** formed by thermal rearrangement during preparation and purification, or that **7a** is formed as an intermediate in the hydrolytic process. Preliminary infrared and nmr data, although not completely definitive, indicate that the compound is a mixture of **7** and **7a,** with **7a** the predominant form. Strong absorption was found at 1690 cm.$^{-1}$ (C=N stretch) which was absent in the infrared spectra of the corresponding O-(methylcarbamoyl)oxime (**31**) or p-nitroacetophenone O-(diethyl phosphoryl)oxime (**13**), both of which gave the respective oxime upon hydrolysis. Nmr spectra of **7** (or **7a**) showed two

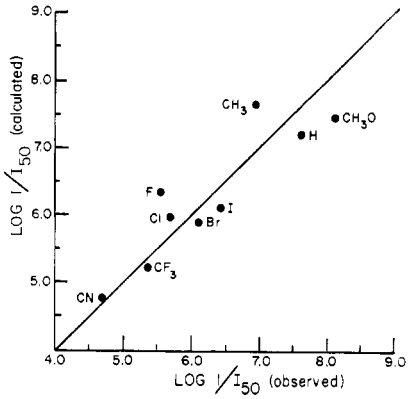


Figure 5. Correlation between observed I_{50} and I_{50} calculated from F and R values according to Equation 6 for p-substituted acetophenone oxime diethyl phosphates

peaks separated by 14 c.p.s. near τ7.8 (α-methyl protons) and two peaks separated by 4 c.p.s. near τ6.2 (p-OCH_3 protons). Since there is no reason for the α-methyl or p-OCH_3 hydrogens to give doublets, it seems likely that the peaks originate from a mixture of **7** and **7a.** The fact that both alkaline hydrolysis rates and anticholinesterase activity are linearly correlated with the electron-donating tendency of the substituent indicates that the compounds represented in Equations 2 and 6 (Figures 2 and 5) contain amounts of rearranged products proportional to hydrolysis constants or anticholinesterase activity. This point needs verification, and intensive work on the rearrangement of oxime phosphates currently is in progress.

Analysis of the m-substituted acetophenone O-(diethyl phosphoryl)oximes gave unusual results. No significant correlation was found between anticholinesterase activity and electronic effects alone but good correlation was obtained with Hansch's π constants. Equations 7 and 8 provided best fit to the data for the five m-substituted phosphates in Table I.

$$\log 1/I_{50} = 5.46\pi^2 - 8.79\pi + 7.02 \qquad r = 0.92 \tag{7}$$

$$\log 1/I_{50} = 3.98\pi^2 - 7.29\pi - 1.33F + 7.46 \qquad r = 0.99 \tag{8}$$

The inclusion of the π term in Equations 7 and 8, let alone π^2, is difficult to explain. According to Hansch and Fujita (1964), π^2 is the highest order of π necessary to account for partitioning in a multiphase biological system. The π terms in the two equations suggest that enzyme-inhibitor interaction through hydrophobic bonding plays a dominant role in the inhibition process. Why this series is different from the other, particularly the m-substituted carbamates, is unexplainable. According to Equation 7, m-substituents with large negative π values should be potent anticholinesterases. Inclusion of the F term in Equation 8 improved the correlation coefficient r from 0.92 to 0.99. However, because of the small number of compounds, the addition of F was not significant at the 0.05 level of probability.

Unlike the α-substituted benzaldehyde O-(methylcarbamoyl)oximes, the relationship between structure and anticholinesterase activity of the corresponding α-substituted benzaldehyde O-(diethyl phosphoryl)oximes (compounds **19** to **25**) was not clear-cut. Examination of the data in Table I shows no obvious relationship, owing mainly to the high anticholinesterase activity of α-isopropyl, **20,** and α-methyl,

1, compared to the ethyl, **19,** and *n*-butyl, **21,** analogs. Possibly, this large difference (10^4-fold) in anticholinesterase activity may be caused by effects due to *syn*- and *anti*-isomerism. However, in the single case where isomers were separated with the carbamates, compounds **46** and **47,** the difference in anticholinesterase activity was only 2-fold.

Setting aside the data for **1** and **20,** regression analysis gave the following equation for the five remaining α-substituted benzaldehyde *O*-(diethyl phosphoryl)oximes.

$$\log 1/I_{50} = 4.54 + 3.95F + 4.11R \qquad r = 0.92 \qquad (9)$$

A noteworthy point in this equation is the positive values for the coefficients of F and R. Evidently, α-substituted benzaldehyde *O*-(diethyl phosphoryl)oximes are unlike ring-substituted *O*-(diethyl phosphoryl)oximes in that electron-attracting substituents produced stronger anticholinesterases. However, this relationship is tempered by the exceptions found in **1** and **20.**

Insect Toxicity. In spite of the generally strong anticholinesterase properties exhibited by the *O*-(diethyl phosphoryl)oximes in Table I, relatively few of the compounds were toxic to the house fly and mosquito larvae. The most toxic compound in the series was *O*-(diethyl phosphoryl) α-cyanobenzaldoxime, **23,** which was expected because of the potent insecticidal properties of the corresponding thionate ester (Bayer 77488). Phosphates showing moderate toxicity were *o*-CH_3 (**2**) and *p*-OCH_3 (**7**) ring substituted esters and α-i-C_3H_7 (**20**) and α-CF_3 substituted esters. No correlation was found between toxicity to house flies and anticholinesterase activity. However, with the α-substituted benzaldehyde *O*-(diethyl phosphoryl)oximes which showed measurable toxicity to mosquito larvae (compounds **1, 20, 23,** and **24**) excellent correlation was obtained between toxicity and cholinesterase inhibition. Regression analysis produced Equation 10.

$$\log 100\ LC_{50} = 14.93 - 1.67 \log 1/I_{50} \qquad r = 0.98 \qquad (10)$$

Inclusion of Taft's polar substituent constant produced Equation 11.

$$\log 100\ LC_{50} = 16.37 + 0.297\ \sigma^* - 1.87 \log 1/I_{50} \qquad r = 0.99 \qquad (11)$$

The values for the predicted LC_{50} using Equation 11 *vs.* observed LC_{50} are plotted in Figure 6.

Except for the α-CF_3-benzaldehyde *O*-(methylcarbamoyl)-oxime (**50**), all of the carbamates in Table II were nontoxic to house flies when used alone. The use of piperonyl butoxide as a synergist increased toxicity to the house fly by substantial amounts in most cases, suggesting that the carbamates are readily detoxified by the house fly. Although no correlation was found between anticholinesterase activity and synergized LD_{50} to house flies with the ring-substituted *O*-(methylcarbamoyl)oximes significant correlation was obtained with the α-substituted benzaldehyde *O*-(methylcarbamoyl)oximes (compounds **26, 43** to **51**). Regression analysis gave the equation below.

$$\log LD_{50} = 4.09 - 0.535 \log 1/I_{50} \qquad n = 9 \qquad r = 0.82 \qquad (12)$$

The relationship between LD_{50} calculated according to Equation 12 and synergized LD_{50} to the house fly is shown in Figure 7. The plot, although not as satisfactory as the other figures, shows a direct relationship between cholinesterase inhibition and toxicity when detoxication rates are minimized by piperonyl butoxide.

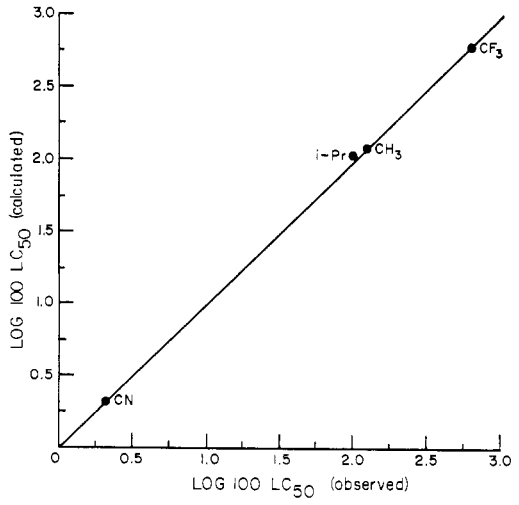


Figure 6. Correlation between observed LC_{50} to mosquito larvae and LC_{50} calculated from σ^* and I_{50} values according to Equation 9 for α-substituted benzaldoxime diethyl phosphates

Excellent correlation was obtained for the α-substituted benzaldehyde *O*-(methylcarbamoyl)oximes between anticholinesterase activity and toxicity to mosquito larvae in those cases where finite values of LC_{50} were obtained. Regression analysis for data from compounds **44, 46, 47, 48,** and **51** gave Equation 13 with a correlation coefficient of 0.99.

$$\log 10\ LC_{50} = 4.46 - 0.59 \log 1/I_{50} \qquad (13)$$

The preceding correlations obtained by multiple regression analysis show that the relationship between the structure of the *O*-(diethyl phosphoryl)oximes and *O*-(methylcarbamoyl)-oximes with chemical reactivity and biological activity does not follow a simple pattern and each series of compounds must be handled separately. The results from the limited amount of data in this investigation indicate that anti-

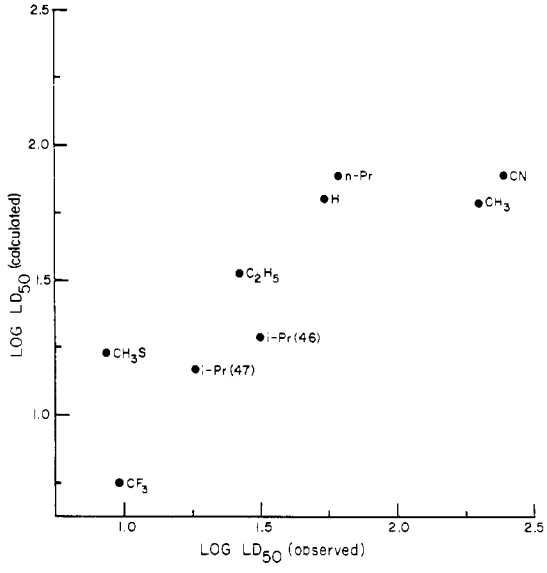


Figure 7. Correlation between observed LD_{50} to house flies and LD_{50} calculated from I_{50} values according to Equation 12 for α-substituted benzaldoxime *N*-methylcarbamates

cholinesterase activity of ring-substituted *O*-(diethyl phosphoryl)oximes and *O*-(methyl-carbamoyl)oximes depends largely on the reactivity of the molecule as estimated by hydrolysis rates and free energy parameters. However, this conclusion is somewhat weakened by the strong effect of π in the anticholinesterase activity of the *m*-substituted acetophenone *O*-(diethyl phosphoryl)oximes. With the α-substituted benzaldehyde *O*-(methylcarbamoyl) oximes, Hansch's π constant has an effect equal to field effects in determining anticholinesterase activity, suggesting strong interaction between the α-substituent and the anionic site of the enzyme in the complexing stage. With the corresponding α-substituted benzaldehyde *O*-(diethyl phosphoryl)oximes, however, π apparently has no effect in improving the correlation between cholinesterase inhibition and electronic effects, indicating that interaction between the α-substituent and anionic site is not important in the inhibition process with these compounds.

Although most of the compounds in Tables I and II were poor in insecticidal activity, the outstanding toxicity of α-cyanobenzaldehyde *O*-(diethyl phosphoryl)oxime, **23,** and moderate toxicity of α-trifluoromethylbenzaldehyde *O*-(diethyl phosphoryl)oxime, **24,** and *O*-(methylcarbamoyl)oxime, **50,** and others justifies further examination of phosphoryl- and carbamoyloximes. Additional work is currently in progress.

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