high molecular weight and also to increasing the molecular weight of many types of polymers having free amino and carboxyl groups.[12]

Experimental

I. Preparation of Starting Polypeptides.—A. Poly-γ-benzyl-L-glutamate (sample 474).—2.0 g. of γ-benzyl-L-glutamate-N-carboxy anhydride[3b] was added to 50 cc. of freshly distilled dioxane. To this solution was added 183 cc. of 0.416 *N* NaOH at −30°. This corresponds to an anhydride:initiator ratio (A/I) of 100. The stoppered flask was allowed to stand at room temperature for 2 hr. during which time the solution became viscous.[13] The polymer was isolated by pouring the above solution into approximately seven times its volume of 95% ethanol which contained sufficient HCl to neutralize the initiator. It was then filtered, washed with anhydrous ether, dried *in vacuo* and stored at 2°. The yield was quantitative; $\eta_{sp/c}$ (*c* 0.2%, in DCA) = 1.22.

Samples number 480 and 483 were prepared using $NaOCH_3$ in benzene–methanol (3:1) as initiator for the polymerization.[3b]

B. Poly-ε-carbobenzyloxy-L-lysine was prepared from the corresponding NCA[14] in the manner described above.

C. Poly-L-proline was prepared from L-proline NCA[15] using $NaOCH_3$ initiation.

II. Preparation of High Molecular Weight Polypeptides.—The following are typical procedures.

A. In Organic Solvents.—0.2 g. of poly-γ-benzyl-L-glutamate ($\eta_{sp/c}$ = 1.22) was dissolved in 20 cc. of dioxane and 0.1 g. of dicyclohexylcarbodiimide was added which dissolved immediately. The reaction mixture was allowed to stand at room temperature for 24 hr. during which time the solution became more viscous. The polymer then was precipitated by pouring the reaction mixture into 100 cc. of 95% ethanol; the dicyclohexylurea and dicyclohexylcarbodiimide is soluble in the ethanol whereas the polymer is insoluble. The polymer then was dried at 100° *in vacuo* for 12 hr. The yield was quantitative: $\eta_{sp/c}$ = 4.82 (*c* 0.2%, in DCA).

B. Aqueous Solutions.—0.028 g. of the starting poly-L-proline ($\eta_{sp/c}$ (*c* 0.2%, in DCA) = 0.18) was dissolved in 5 cc. of distilled water, and 0.020 g. of 1-ethyl-3-[2-morpholinyl-(4)-ethyl]-carbodiimide metho-*p*-toluenesulfonate was added. The reaction mixture was allowed to stand at room temperature for 24 hr. The solvent was removed by lyophilization and the polymer was extracted with absolute ethanol for 2 hr. to remove the substituted urea and the unreacted carbodiimide. After drying at 100° *in vacuo* $\eta_{sp/c}$ (*c* 0.14%, in DCA) = 0.32.

Acknowledgments.—We are pleased to acknowledge the support of this work by the Office of the Surgeon General, Department of the Army, and the valuable aid of Dr. G. D. Fasman and Mr. K. Norland in some of the preparative work.

(12) *Cf.* J. C. Sheehan and J. J. Hlavka, THIS JOURNAL, **79**, 4258 (1957).

(13) In the poly-γ-benzyl-L-glutamate preparations the polymerization was stopped soon after the consumption of the NCA and the polymers were stored at 2° in order to minimize the formation of pyrrolidones at the terminal amino acid residues (W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950)) which would interfere with the subsequent carbodiimide condensations.

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BOSTON 15, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF ENTOMOLOGY OF THE UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION]

The Effect of Structure on the Reactivity of Alkylphosphonate Esters[1,2]

BY T. R. FUKUTO AND R. L. METCALF

RECEIVED MARCH 10, 1958

A number of new ethyl *p*-nitrophenyl alkylphosphonates were prepared and the effect of the alkyl moiety on the lability of the phosphorus–O–*p*-nitrophenyl bond as measured by alkaline hydrolysis was examined. It was found that the rate of hydrolysis of these alkylphosphonates to *p*-nitrophenol and ethyl alkylphosphonic acid, in general, decreased with increase in alkyl chain length. Compared to the straight chain compounds, branching in the 1- and 2-positions greatly decreased the hydrolysis rate. The rate of reaction of these compounds with insect cholinesterase also was measured and compared with the hydrolysis rate. Many of these compounds showed high toxicity to the common house fly, *Musca domestica* L., and degree of toxicity was parallel to cholinesterase inhibition.

The toxicity of certain organophosphorus compounds to mammals and insects has generally been associated with the inhibition of the cholinesterase (ChE) enzymes. The inhibition of these esterases by organophosphorus compounds has clearly been demonstrated to be the result of an actual chemical reaction between the enzyme and the phosphorus compound.[3] The phosphorylated enzyme thus produced is no longer able to catalyze the hydrolysis of certain esters.

The inhibition of erythrocyte cholinesterase by diethyl *p*-nitrophenyl phosphate (para-oxon) and some of its analogs has been shown to proceed with pseudo first-order kinetics.[4] The bimolecular rate constants for this inhibition were determined for the various para-oxon analogs, and these values paralleled the rates of hydrolysis of these phosphates in water. More recently[5] it was demonstrated from the study of a large series of diethyl-substituted phenyl phosphates that the inhibition of fly-head cholinesterase by these compounds was related to the effect of the substituent on the lability of the P–O–phenyl bond as measured by Hammett's σ constants, shifts in P–O–phenyl stretching frequencies, and hydrolysis rates.

Although the phosphate esters and their sulfur analogs have been widely examined as inhibitors of cholinesterase and evaluated as insecticides, relatively little has been published on the activity of phosphonate esters in spite of the fact that some of the most potent inhibitors are derivatives of phosphonate esters. 3,3-Dimethyl-2-butyl methylphos-

(1) Paper No. 1052, University of California Citrus Experiment Station, Riverside, Calif.

(2) Supported in part by a research grant from the Monsanto Chemical Company, St. Louis, Mo.

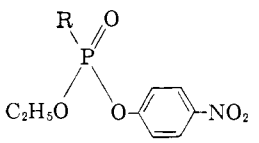
(3) T. R. Fukuto, in "Advances in Pest Control Research," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1957, pp. 147–192.

(4) W. N. Aldridge and A. N. Davison, *Biochem. J.*, **51**, 62 (1952).

(5) T. R. Fukuto and R. L. Metcalf, *J. Agr. Food Chem.*, **4**, 930 (1956).

phonofluoridate (soman) and isopropyl methylphosphonofluoridate (sarin) are two of the most powerful anticholinesterase poisons known.[6]

As an obvious extension to our studies regarding the structure–activity relationships of organophosphorus anticholinesterases, it was decided to investigate the effect of variations in the alkyl group R on the lability of the P–O–*p*–$C_6H_4NO_2$ bond in a series of ethyl *p*-nitrophenyl alkylphosphonates. This particular series of compounds was selected for two reasons, (1) the structural similarity of these compounds to para-oxon and



(2) the relative ease with which some of the properties of these compounds may be examined by spectrophotometry.

Experimental

Diethyl Alkylphosphonate.—In most cases the diethyl alkylphosphonate was prepared by application of the Michaelis–Arbuzov reaction,[7,8] in which equivalent quantities of triethyl phosphite and alkyl halide are heated to give diethyl alkylphosphonate and ethyl halide. The apparatus used to carry out this reaction is described by Kosolapoff.[7]

Triethyl phosphite was obtained from the Virginia–Carolina Chemical Corporation, Richmond, Va., and was used without any purification. Iodomethane, 1-iodopropane, 1-iodobutane, 1-bromopentane, 1-bromohexane, 1-bromo-3-methylbutane, 1-bromo-3-chloropropane and benzyl chloride were Eastman white-label materials and used without further purification.

Diethyl 4-Methylpentylphosphonate.—A mixture of 11 g. of 1-bromo-4-methylpentane[9] and 11 g. of triethyl phosphite was heated until the theoretical quantity of ethyl bromide (7.2 g.) was collected. Distillation of the residual liquid yielded 81% of product, b.p. 68–70° (0.3 mm.), $n^{30}D$ 1.4224.

Anal. Calcd. for $C_{10}H_{23}O_3P$: C, 54.03; H, 10.41. Found: C, 54.18; H, 11.13.

Diethyl 4,4-dimethylpentylphosphonate was prepared in the manner described above from 1-bromo-4,4-dimethylpentane[10] and triethyl phosphite in 80% yield, b.p. 78–80° (0.3 mm.), $n^{30}D$ 1.4252.

Anal. Calcd. for $C_{11}H_{25}O_3P$: C, 55.91; H, 10.66. Found: C, 55.82; H, 11.04.

Diethyl 3-chloropropylphosphonate was prepared in the same manner described above from 1-bromo-3-chloropropane and triethyl phosphite in 45% yield, b.p. 80–82° (0.1 mm.), $n^{30}D$ 1.4426.

Anal. Calcd. for $C_7H_{16}ClO_3P$: C, 39.18; H, 7.52. Found: C, 39.33; H, 7.85.

Diethyl isopropylphosphonate,[11] diethyl cyclohexylphosphonate[12] and diethyl phenylphosphonate[8] were prepared from isopropylphosphonic dichloride,[13] cyclohexylphosphonic dichloride[14] and phenylphosphonic dichloride (obtained through the courtesy of the Victor Chemical Co., Chicago Heights, Ill.), respectively, by treatment with ethyl alcohol and pyridine, according to the method of Hudson and Keay.[15]

Diethyl Isobutylphosphonate.—Diisobutyl isobutylphosphonate[16] was converted to isobutylphosphonic dichloride[17] according to the procedure of Kabachnik and Rossiiskaya.[18] Treatment of the dichloride with ethyl alcohol and pyridine gave diethyl isobutylphosphonate, b.p. 90–91° (8 mm.), $n^{30}D$ 1.4172.

Anal. Calcd. for $C_8H_{19}O_3P$: C, 49.47; H, 9.92. Found: C, 49.44; H, 10.33.

Ethyl Alkylphosphonochloridate.—With the exception of ethyl *t*-butylphosphonochloridate, all ethyl alkylphosphonochloridates were prepared by the reaction between equimolar quantities of diethyl alkylphosphonate and phosphorus pentachloride in the cold.[18] In all cases the formation of varying amounts of dichloride was unavoidable and it could not be separated by distillation; therefore, the products were not characterized, but were carried on through to the ethyl *p*-nitrophenyl alkylphosphonate.[19] Ethyl *t*-butylphosphonochloridate was prepared from *t*-butylphosphonic dichloride by reaction with an equivalent quantity of sodium ethylate in ethyl alcohol. The boiling points of the crude uncharacterized ethyl alkylphosphonochloridates are given in Table I. The yields of crude material ranged from 60–70%.

Ethyl *p*-Nitrophenyl Alkylphosphonate.—The ethyl *p*-nitrophenyl alkylphosphonates were prepared by the reaction of equivalent quantities of sodium *p*-nitrophenoxide and ethyl alkylphosphonochloridate in an inert solvent. The following procedure described for the synthesis of ethyl *p*-nitrophenyl ethylphosphonate is typical.

Ethyl *p*-Nitrophenyl Ethylphosphonate.—To a 200-ml. 3-necked flask equipped with stirrer, dropping funnel and distillation head was added 14.1 g. of sodium *p*-nitrophenoxide. Approximately 100 ml. of toluene was added, and toluene was removed by distillation until the mixture in the flask was dry, as indicated by the distillate. The mixture was allowed to cool to room temperature, the distillation head was replaced with a condenser fitted with a calcium chloride drying tube, and 13.7 g. of ethylphosphonochloridate was added dropwise with stirring. After the addition was completed, the mixture was stirred and heated at reflux for an additional 2 hours. The mixture was allowed to cool to room temperature and was then washed four times with cold 5% sodium hydroxide and four times with water and dried over anhydrous sodium sulfate. The excess toluene was removed under water aspirator pressure and the yellow oil remaining was distilled, b.p. 145–146° (0.2 mm.). The yield of ethyl *p*-nitrophenyl ethylphosphonate was 7.0 g. A large portion of the product which came over as the forerun, b.p. up to 145° (0.2 mm.), was contaminated with *p*-nitrophenol.

The reaction between ethyl *t*-butylphosphonochloridate and sodium *p*-nitrophenoxide was carried out in ethyl alcohol instead of the usual solvent toluene. No reaction occurred in toluene. The physical properties of the ethyl *p*-nitrophenyl alkylphosphonates are summarized in Table I.

Ethyl 2-(Ethylthio)-ethyl *n*-Propylphosphonothiolate (XVIII).—To 1.5 g. of powdered sodium in toluene was added 8 g. of 2-(ethylthio)-ethanethiol.[20] The mixture was stirred and heated until the sodium disappeared. Ethyl *n*-propylphosphonochloridate (11.2 g.) was added dropwise and the mixture was heated at reflux for 2 hours. The toluene solution was washed three times with cold 5% sodium hydroxide and four times with water; it was then dried over anhydrous sodium sulfate and the product was distilled. The fraction distilling at 98–100° (0.05 mm.) was collected. The yield was 10 g.

Anal. Calcd. for $C_9H_{21}O_2PS_2$: C, 42.16; H, 8.26. Found: C, 42.25; H, 8.15.

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(19) It has been reported recently that excellent yields of pure alkyl ethylphosphonochloridates are obtained when this reaction is carried out in carbon tetrachloride; *cf.* A. I. Razumov, O. A. Mukhacheva and E. A. Markovich, *Zhur. Obshchei Khim.*, **27**, 2389 (1957).

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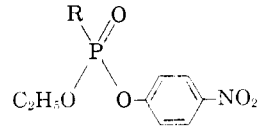
TABLE I

PHYSICAL CONSTANTS OF ETHYL *p*-NITROPHENYL ALKYLPHOSPHONATES



	R	B.p. of intermediate ethyl alkylphosphonochloridate °C.	Mm.	B.p. °C.	Mm.	$n^{30}D$	d^{30}_4	Formula	Analyses, % Calcd.	Found
I	CH_3[a]	82–88	25	125	0.1	1.5240	1.2782	$C_9H_{12}NO_5P$	C 44.09	C 43.71
									H 4.93	H 5.59
II	C_2H_5[b]	80–86	15	145–146	.2	1.5195	1.2446	$C_{10}H_{14}NO_5P$	C 46.33	C 46.86
									H 5.34	H 5.57
III	*n*-C_3H_7	84–92	12	133–135	.1	1.5137	1.2184	$C_{11}H_{16}NO_5P$	C 48.36	C 48.00
									H 5.90	H 6.46
IV	*n*-C_4H_9	85–95	8	140–142	.1	1.5134	1.1986	$C_{12}H_{18}NO_5P$	C 50.16	C 50.22
									H 6.32	H 6.43
V	*n*-C_5H_{11}	104–110	6	140–144	.05	1.5080	1.1632	$C_{13}H_{20}NO_5P$	C 51.82	C 50.85
									H 6.69	H 7.01
VI	*n*-C_6H_{13}	110–112	9.5	146–150	.05	1.5029	1.1359	$C_{14}H_{22}NO_5P$	C 53.30	C 53.20
									H 7.03	H 7.84
VII	*i*-C_3H_7	86–96	25	148	.5	1.5137	1.2259	$C_{11}H_{16}NO_5P$	C 48.36	C 47.96
									H 5.90	H 6.11
VIII	*i*-C_4H_9	90–98	15	157–159	.5	1.5114	1.1899	$C_{12}H_{18}NO_5P$	C 50.16	C 50.18
									H 6.32	H 6.69
IX	*i*-C_5H_{11}	96–110	7	142	.05	1.5079	1.1654	$C_{13}H_{20}NO_5P$	C 51.82	C 51.56
									H 6.69	H 6.87
X	*i*-C_6H_{13}	110–112	5	170	.2	1.4928	1.1256	$C_{14}H_{22}NO_5P$	C 53.32	C 52.61
									H 7.03	H 7.66
XI	*t*-C_4H_9	70–72	5	120	.03	1.5100	1.1767	$C_{12}H_{18}NO_5P$	C 50.16	C 50.20
									H 6.32	H 6.52
XII	4,4-Dimethylpentyl	110–114	4	140[c]	.1	1.5012	1.1229	$C_{15}H_{24}NO_5P$	C 54.71	C 54.46
									H 7.35	H 7.65
XIII	Cyclohexyl	100–102	2	140[c]	.05	1.5286	1.2097	$C_{14}H_{20}NO_5P$	C 53.67	C 53.64
									H 6.75	H 6.89
XIV	Phenyl	106–110	2	164–166	.05	1.5621	1.2736	$C_{14}H_{14}NO_5P$	C 54.73	C 54.76
									H 4.59	H 4.71
XV	Benzyl	120–125	6	170[c]	.03	1.5568	1.2584	$C_{15}H_{16}NO_5P$	C 56.07	....[d]
									H 5.02	....
XVI	$ClCH_2CH_2CH_2$	86–90	0.5	175[c]	.05	1.5340	1.3334	$C_{11}H_{15}ClNO_5P$	C 42.93	C 42.57
									H 4.92	H 5.15

[a] This compound was reported by G. Schrader, BIOS Final Report No. 714. However, only the b.p. 178° (1.5 mm.) is reported. [b] A. I. Razumov, E. A. Markovich and O. A. Mukhacheva (*Khim. i Primemenie Fosfororgan. Sodinenii, Akad. Nauk, S.S.S.R.*, Trudy l-oi Konferents., 194 (1955), *C. A.*, **52**, 237 (1958)) report this compound, b.p. 163–165° (0.2 mm.), $n^{20}D$ 1.5242, d^{20}_0 1.2545, and other alkyl *p*-nitrophenyl ethylphosphonates. [c] Distilled in falling-film molecular still. [d] This compound left a dark residue upon combustion and a satisfactory analysis could not be obtained.

Ethyl 2-(ethylthio)-ethyl methylphosphonothiolate (XIX) was prepared in the same manner as the above propyl analog, using 1.2 g. of sodium, 6.5 g. of 2-(ethylthio)-ethanethiol and 7.7 g. of ethyl methylphosphonochloridate. The product distilled at 88–90° (0.05 mm.).

Anal. Calcd. for $C_7H_{17}O_2PS_2$: C, 36.82; H, 7.51. Found: C, 36.44; H, 7.58.

Kinetic Methods.—The hydrolysis rates of the ethyl *p*-nitrophenyl alkylphosphonates were determined colorimetrically in Sorensen's phosphate buffer (*p*H 8.3) at 37.5°. Solutions were made up approximately 4×10^{-5} *M* in ethyl *p*-nitrophenyl alkylphosphonate in phosphate buffer. The amount of *p*-nitrophenoxide ion formed after measured time intervals was estimated colorimetrically in a Beckman DU spectrophotometer at 400 mμ.[21] The rate constant K_{hyd} was calculated from first-order plots of the kinetic data. The values given in Table II represent the average of at least three independent determinations.

The rate of reaction of the ethyl *p*-nitrophenyl alkylphosphonate with the cholinesterase enzyme was determined manometrically at 37.5° by the method described by Aldridge,[22,4] using double-armed Warburg flasks. The reactions were carried out in a mixture consisting of a brei of three homogenized fly heads per ml. of a solution 0.025 *M* in sodium bicarbonate, 0.15 *M* in sodium chloride, 0.04 *M* in magnesium chloride and 0.01 *M* in acetylcholine bromide. The bimolecular rate constant of the reaction between ethyl *p*-nitrophenyl alkylphosphonate and fly-head cholinesterase was calculated by the method of Aldridge.[4] The values given in Table II represent two independent determinations.

The method to determine the 50% inhibition concentrations (I_{50}) of fly-head cholinesterase by phosphonate has been described.[5]

The technique for determination of the contact toxicity (LD_{50}) of the ethyl *p*-nitrophenyl alkylphosphonates to the

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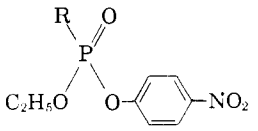
(22) W. N. Aldridge, *Biochem. J.*, **46**, 451 (1950).

TABLE II

RATE CONSTANTS AND TOXICITY DATA FOR ETHYL *p*-NITROPHENYL ALKYLPHOSPHONATE



	R	K_e, l. moles^{-1} min.$^{-1}$	K_{hyd}, min.$^{-1}$	I_{50} (molar concn.)	LD_{50} female house fly (γ per g. fly)
I	CH_3	7.6×10^6	2.42×10^{-3}	4×10^{-8}	1.0
II	C_2H_5	1.5×10^7	5.06×10^{-4}	1.3×10^{-8}	1.2
III	n-C_3H_7	1.0×10^7	4.17×10^{-4}	1.0×10^{-8}	2.0
IV	n-C_4H_9	4.0×10^6	4.23×10^{-4}	1.1×10^{-8}	2.4
V	n-C_5H_{11}	8.9×10^5	3.62×10^{-4}	6.8×10^{-8}	12
VI	n-C_6H_{13}	6.9×10^5	3.56×10^{-4}	6.1×10^{-8}	62
VII	i-C_3H_7	1.1×10^5	1.07×10^{-4}	5×10^{-7}	28
VIII	i-C_4H_9	3.9×10^5	2.34×10^{-4}	3.3×10^{-7}	9
IX	i-C_5H_{11}	4.9×10^6	2.45×10^{-4}	1.1×10^{-8}	7
X	i-C_6H_{13}	2.0×10^6	3.62×10^{-4}	3.1×10^{-8}	40
XI	t-C_4H_9	6.7	3.2×10^{-6}[a]	$>3 \times 10^{-3}$	>5000
XII	4,4-Dimethylpentyl	6.6×10^5	3.41×10^{-4}	1.1×10^{-7}	57
XIII	Cyclohexyl	6.1×10^4	3.07×10^{-5}	4×10^{-7}	>500
XIV	Phenyl	1.8×10^6	2.64×10^{-3}	3×10^{-8}	2.8
XV	Benzyl	1.9×10^5	7.05×10^{-4}	3×10^{-7}	>500
XVI	$ClCH_2CH_2CH_2$	5.9×10^5	7.53×10^{-4}	1.3×10^{-7}	8.5
XVII	OC_2H_5[b]	2.7×10^7	6.14×10^{-5}	2.6×10^{-8}	1.2

[a] The hydrolysis was extremely slow (1.5% in 2 weeks) and an approximate value is given. [b] XVII (para-oxon) is given so that the data of the phosphonates may be compared with those of a phosphate of similar structure.

common house fly, *Musca domestica L.*, has been described.[23] The LD_{50} values in Table II were obtained from log dosage *vs.* probit mortality plots, and each value is the average of at least three complete replications. The standard deviation of 3 replicate determinations by the methods employed was ±30%.

Results and Discussion

Alkaline hydrolyses of the ethyl *p*-nitrophenyl alkylphosphonates were carried out in Sorensen's phosphate buffer at *p*H 8.3 at 37.5° by measuring the rate of *p*-nitrophenoxide ion formation. The calculated pseudo first-order rate constants (K_{hyd}) listed in Table II show that the nature of the alkyl group R in ethyl *p*-nitrophenyl alkylphosphonate greatly influences the stability of the compound to alkaline hydrolysis. The cleavage of the P–O–*p*-nitrophenyl bond probably occurs by nucleophilic attack on the phosphorus atom by a nucleophilic agent (OH^-, $HPO_4^=$), thus displacing *p*-nitrophenoxide ion.

It can be seen from the values given that although there is a large difference in the hydrolysis rate between the methyl- (I) and ethylphosphonate (II), further increases in chain length in the normal series have relatively little effect on the rate. This is in disagreement with the values found by Hudson and Keay[15] for the alkaline hydrolysis of the diethyl and diisopropyl esters of methyl-, ethyl-, *n*-propyl- and *n*-butylphosphonic acids. The relative rates of reaction of these compounds with hydroxide ion in water are given as follows: for diisopropyl alkylphosphonate, methyl 1, ethyl 0.16, *n*-propyl 0.062, *n*-butyl 0.039, *t*-butyl 0.002; and for diethyl alkylphosphonate, methyl 1, ethyl 0.50, *n*-butyl 0.10, phenyl 1.7. These values show a consistent drop in rate with chain length increase for the straight chain compounds. A further comparison may be made with esters of carboxylic acids. The relative rates of alkaline hydrolysis of the aliphatic carboxylic ester R–$COOC_2H_5$ in aqueous 88% ethyl alcohol, where R is varied, are given as: methyl 1, ethyl 0.470, *n*-propyl 0.274, *n*-butyl 0.262, isopropyl 0.100, *t*-butyl 0.0105, phenyl 0.102 and benzyl 1.322.[24,25] It appears from these data that the relative rates of hydrolysis of ethyl-, *n*-propyl-, *n*-butyl-, isopropyl- and *t*-butylphosphonate are in closer agreement with those of the carboxylic esters.

Although increases in chain length from two to six carbon atoms in the straight chain compounds had little effect on the hydrolysis rate, branching, particularly on the 1-carbon atom, greatly reduced the hydrolysis rate. The relative hydrolysis rates of ethyl *p*-nitrophenyl isopropylphosphonate (VII), cyclohexylphosphonate (XIII) and *t*-butylphosphonate (XI) compared to I are 0.044, 0.013 and 0.0013, respectively. By analogy with carboxylic esters the decrease in rate probably is due to a steric effect. Branching on the 2-, 3- and 4-carbons, however, had little effect on the hydrolysis rates although steric factors still appear to be operating in view of the slight increases in rate as the methyl group was placed farther from the phosphorus atom. The hydrolysis rate of the phenylphosphonate (XIV) was 1.1 times faster than I. This agrees with relative rate found by Hudson and Keay[15] for diethyl methylphosphonate and diethyl phenylphosphonate and is expected in view of the electron-sink properties of the benzene ring. As one might suspect, the presence of a halogen atom in the chain increased the rate (compare *n*-propyl (III) and 3-chloropropyl (XVI)).

The rate of reaction between the ethyl *p*-nitrophenyl alkylphosphonates and fly-head cholinesterase was measured manometrically at 37.5°.

(23) R. L. Metcalf and R. B. March, *J. Econ. Entomol.*, **46**, 288 (1953).

(24) K. Kindler, *Ann.*, **452**, 90 (1927).

(25) K. Kindler, *Ber.*, **69B**, 2792 (1936).

The calculated bimolecular rate constants (K_e) are given in Table II. The values show that in general the rate of enzyme inhibition parallels the hydrolysis rate. It has been shown by Aldridge and Davison[4] that a linear relationship is obtained when the log of the inhibition constant is plotted against the log of the hydrolysis constant for a series of diethyl-substituted phenyl phosphates, implying that the mechanism of the reaction between nucleophilic agents and enzyme with phosphate is the same. A similar plot with the phosphonates (Fig. 1) shows that although there is a general trend the relationship is not linear.

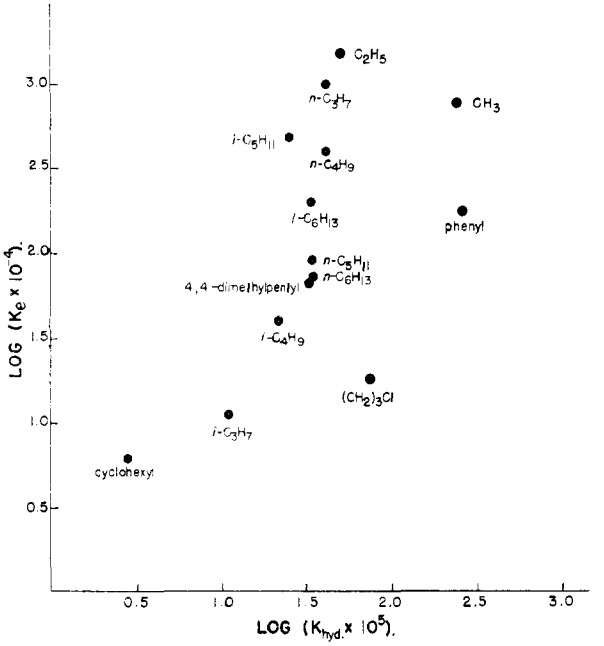


Fig. 1.—A plot of log cholinesterase inhibition constant K_e *versus* log hydrolysis constant K_{hyd}.

Compounds I, II and III showed the highest anticholinesterase activity. As the chain length is increased from 3 to 6 carbon atoms, the rate of inhibition of cholinesterase drops rapidly for the straight-chain compounds. It is apparent from Fig. 1 that branching on the 1- or 2- carbon atoms also greatly lowers the inhibition rate. The low rate of inhibition of cholinesterase by these compounds is probably due to steric factors. The stability of VII, XIII and XI to basic hydrolysis has been attributed to steric factors, and similar considerations probably hold true for the reaction between enzyme and phosphonate. The tenfold lowering of the inhibition rate by branching on the 2-carbon atom in spite of similar alkaline hydrolysis rates (compare *n*-butyl (IV) and isobutyl (VIII)) suggests that steric fit at the reactive site on the enzyme surface must also be considered. It appears from the relatively high inhibition rates by isopentylphosphonate (IX) and isohexylphosphonate (X) compared to their straight chain analogs, that methyl groups at the 3- and 4-carbon atoms enhance the rate. This suggests that branching is desirable, but at least 3 or 4 carbons removed from the phosphorus atom. It was reasoned from these findings that a compound whose over-all spatial configuration

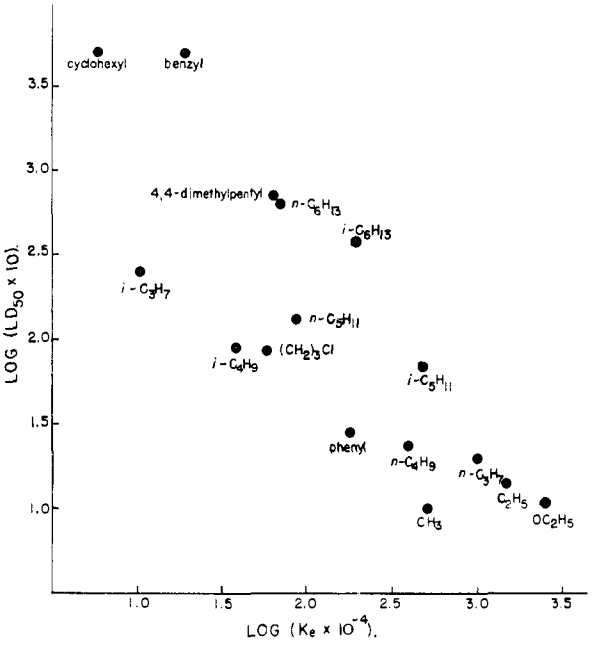
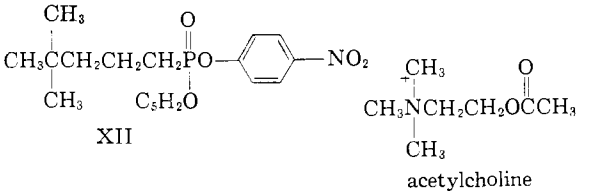


Fig. 2.—A plot of log cholinesterase inhibition constant K_e *versus* log LD_{50} to the common house fly.

most closely resembled the natural cholinesterase substrate acetylcholine might show unusually high anti-cholinesterase activity. For this reason the 4,4-dimethylpentylphosphonate (XII) was prepared and examined. Its spatial relationship with acetylcholine is shown below. This compound was disappointingly weak as an inhibitor, showing about one-third the activity of the isohexyl analog.



The idea of steric attraction to enhance activity warrants additional study, however, and further work is currently in progress. The low inhibition rate by the 3-chloropropylphosphonate was surprising, but not entirely unexpected. One might expect that the chlorine atom serving as a region of high electron density at a rather strategic distance from the phosphorus atom would be repelled by the anionic site[26] on the enzyme surface.

All compounds were examined for their toxicity against the common house fly, *Musca domestica* L., and the amounts which cause 50% mortality after 24 hours (LD_{50}) are given in Table II. Many of the compounds, particularly I, II, III and IV were extremely toxic to the house fly. In a previous paper,[21] it was shown that with substituted N-methylcarbamates, a direct relationship exists between the log of the Michaelis inhibition con-

(26) The active center of the acetylcholinesterase surface is regarded as consisting of two primary regions, (a) a negatively charged site which facilitates enzymic activity by attracting, binding and orienting cationic substrates by virtue of coulombic attraction and van der Waals dispersion forces, and (b) an "esteratic" site which is responsible for hydrolytic activity. For further discussion see I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).

stant and log median lethal concentration to the greenhouse thrips. A similar plot with ethyl *p*-nitrophenyl alkylphosphonates of log LD_{50} (house fly) against log K_e is presented in Fig. 2. It can be seen that the compounds which showed the highest enzyme inhibition rates were most toxic.

Since it is apparent that some phosphonate esters are probably equally as effective as insecticides as phosphate esters of similar structure (compare I, II and III with XVII) it was decided to prepare and study the properties of two phosphonate analogs of the well-known systemic insecticide isoSystox (O,O-diethyl 2-(ethylthio)-ethyl phosphorothiolate). The two compounds prepared were ethyl 2-(ethylthio)-ethyl *n*-propylphosphonothiolate (XVIII) and ethyl 2-(ethylthio)-ethyl methylphosphonothiolate (XIX).[27] Compound XIX was selected because its corresponding *p*-nitrophenyl ester (I) showed the highest toxicity to the house fly, and compound XVIII was selected because its corresponding *p*-nitrophenyl ester III showed relatively high insect toxicity and was more stable to alkaline hydrolysis than I or II. Both XVIII and XIX were examined for their systemic activity in young cotton plants and were found to be extremely active. This work is still in progress, but the preliminary data show that XVIII may be even better as a systemic insecticide than isoSystox. Insect toxicological studies of these compounds will be reported in detail elsewhere.

(27) During the preparation of this manuscript the synthesis of ethyl 2-(ethylthio)-ethyl ethylphosphonothiolate was reported by H. S. Aaron, T. M. Shryne and J. J. Miller, THIS JOURNAL, **80**, 456 (1958).

Acknowledgments.—The authors thank Miss Marianne Y. Winton, Miss Patricia A. Roberts and Mr. John C. Bagger for valuable technical assistance. Semi-microanalyses were carried out by Mr. C. F. Geiger, Ontario, Calif.

RIVERSIDE, CALIF.

[CONTRIBUTION FROM THE DOW CHEMICAL CO., MIDLAND DIVISION]

Synthesis of Some Amino Acid Derivatives of Styrene[1]

BY L. R. MORRIS, R. A. MOCK, C. A. MARSHALL AND J. H. HOWE

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Several vinyl-substituted amino acids have been prepared as intermediates in the synthesis of chelating polymers. Most of the syntheses started with vinylbenzyl chloride and its derivatives. These products retain the polymerizability of styrene as well as the capacity to chelate metal ions. Polymers, both soluble and crosslinked, have been prepared and show interesting properties as chelating resins.

It has often been proposed that introduction of chelating groups into resins would give more selective exchange of various ions than possible with presently available ion exchange resins. Several experimental approaches to synthesis of such chelating resins have given qualitative success, but apparently suffered from poor physical form or indefinite chemical structure of the products. Many of these explorations were made on chemically modified or condensation polymers, wherein crosslinking and homogeneity of structure are difficult to control. Some of the most promising work thus far reported is that by Hale and co-workers in the Teddington Laboratories,[2] who have modified a polystyrene starting material *via* the chloromethylated intermediate commonly used for anion exchange resin synthesis. Although the possibilities and properties of this structure were qualitatively described, the resins did not appear to give practical rates of reaction. A good general review of chelating resins literature is found in the reference by Millar.[3]

In order to avoid many problems exposed in this early work, the synthesis of the requisite monomeric structures, followed by polymerization, was an attractive course. Since exchange resins based on polystyrene have wide-spread applications, the synthesis of some styrene derivatives containing chelating groups was initiated. Also, since amino acids are well known as complexing agents for a number of metal ions, these seemed a logical choice to introduce into a resin.

Vinylbenzyl chloride (I) was a key starting material and was supplied for this work by the Dow Laboratories. This was an attractive intermediate because of the variety of reactions which the benzyl halide structure offered. It has been shown that many reactions of benzyl chloride may be conducted with the vinylbenzyl chloride, provided conditions are not too drastic. Inhibitors against free-radical polymerization are occasionally found necessary to minimize this side reaction. It should be noted here that the starting material is a mixture of isomers, about 80% *para* and 20% *ortho*, and that derivatives therefrom will likely be of similar orientation unless the isomer ratio is altered by purification procedures. In a few cases, pure *p*-vinylbenzyl chloride has been used, but the yields and products were not distinguishable from an isomer mixture in regard to reactions and properties. This was especially true of the amino acid derivatives which did not possess discrete melting points.

Direct alkylation of several amino acids was successfully accomplished in aqueous or partly alcoholic solutions, using alkaline solutions of the amino acids. This may be illustrated by the reaction with iminodiacetic acid. Although this was in part a heterogeneous reaction, the conversion measured by ionic halide was more than 95% com-

(1) Presented in part before the Division of Polymer Chemistry at the 134th National Meeting of the American Chemical Society, Chicago, Ill., September, 1958.

(2) D. K. Hale, *Research*, **9**, 104 (1956).

(3) J. R. Millar, *Chem. Ind.*, **20**, 606 (1957).