Synthesis, Hydrolysis, and Toxicity Data on Substituted Phenacyl *p*-Nitrophenyl Methylphosphonates

C. N. Lieske,[1] J. W. Hovanec,[1] G. M. Steinberg,[1] J. N. Pikulin,[1] W. J. Lennox,[2] A. B. Ash,[3] and P. Blumbergs[3]

A series of four para-substituted phenacyl *p*-nitrophenyl methylphosphonates, $p\text{-}NO_2C_6H_4OP(O)(CH_3)OCH_2C(O)C_6H_4X$, was prepared as follows: I (X = *p*-H-) was prepared by reaction of phenacyl alcohol with *p*-nitrophenyl methylphosphonochloridate. II (X = *p*-CH_3O-), III (X = *p*-Cl-), and IV (X = *p*-NO_2-) were prepared by the reaction of *p*-nitrophenyl methylphosphonic acid with the suitably substituted benzoyldiazomethane. The kinetics of the alkaline hydrolysis of I, II, III, and IV in 0.1*M* tris(hydroxymethyl)aminomethane buffer, pH 7.61, 25.0° C., were determined spectrophotometrically. The half lives ranged from 6 to 180 seconds, and the first-order rate constants fit the Hammett linear free-energy relationship (ρ = 1.43). Simultaneously, the approximate LD_{50} values of these compounds were determined for mice. The toxicity of these compounds shows an inverse relationship to their rates of hydrolysis.

Development of the chemistry of physiologically active organophosphorus compounds has been stimulated by a variety of interests and demands. Foremost has been the need for insecticides to control agricultural pests, and studies on the chemical mechanism of transmission of nervous excitation. In the last decade the mechanisms of the reactions of organophosphorus compounds have been the subject of intensive study (Bruice and Benkovic, 1966). Our particular interest in this area is the study of neighboring group participation in phosphonate ester hydrolysis (Lieske *et al.*, 1966, 1968). A number of other investigators are currently involved in similar studies (Brown and Usher, 1965; Cadogan and Maynard, 1966; Frank and Usher, 1967; Gordon *et al.*, 1964; Witzel *et al.*, 1965; Zioudrou and Schmir, 1963).

Lieske *et al.* (1966) have reported on the effect of a neighboring ketonic group in the alkaline hydrolysis of *p*-nitrophenyl phenacyl methylphosphonate (*I*). In conjunction with continuing studies on the large rate increase in phosphonate ester hydrolysis which results from the incorporation of a suitably placed ketonic group into the molecule, we have investigated the alkaline hydrolysis of *p*-methoxyphenacyl *p*-nitrophenyl methylphosphonate (*II*), *p*-chlorophenacyl *p*-nitrophenyl methylphosphonate (III), and *p*-nitrophenacyl *p*-nitrophenyl methylphosphonate (IV). These are anticholinesterase compounds with possible insecticidal utility. To provide initial toxicity guidelines for personnel handling these compounds, approximate LD_{50} (range-finding) values in Charles River-Swiss mice were determined. The relationship between structure of phosphate and phosphonate esters, their anticholinesterase properties, and toxicity have been examined in great detail (Heath, 1961; Schrader, 1963). The results of our lethality studies suggest that I, II, III, and IV should be handled with due caution.

[1] Physiology Department, Medical Research Laboratory, Edgewood Arsenal, Md. 21010
[2] Organic Chemistry Department, Chemical Research Laboratory, Edgewood Arsenal, Md. 21010
[3] Ash-Stevens, Inc., Detroit, Mich. 48202

Table I. Alkaline Hydrolysis of Title Compounds

Compound	k_{obsd}, Sec.$^{-1}$	$t_{1/2}$, Sec.
I	1.15×10^{-2}	60.2
II	0.38×10^{-2}	182.2
III	2.28×10^{-2}	30.4
IV	11.8×10^{-2}	5.86

The hydrolysis of I in 5.0% aqueous *p*-dioxane (v./v.) at 25.0° C. over the pH range 7.2 to 8.0 in the presence of 0.1*M* tris(hydroxymethyl)aminomethane (Tris) is first order in phosphonate, hydroxide ion, and Tris·Base (Lieske *et al.*, 1966). The alkaline hydrolysis of I, II, III, and IV has now been examined in 5.0% *p*-dioxane–95.0% 0.1*M* Tris buffer (v./v.) of pH 7.61 at 25.0° C. Results are listed in Table I. Excellent first-order plots were obtained to >4 half lives. In each kinetic experiment under the described conditions, the quantity of *p*-nitrophenol formed at t_∞, as measured spectrophotometrically from the absorption of its anion at 400 mμ, corresponded to 1 mole per mole of the methylphosphonate ester.

To explore further the relationship between structure and reactivity in this series of compounds, the data were treated and found to comply with the linear free-energy relationship originally postulated by Hammett (1940) (Figure 1). The sigma values were taken from Hine (1962) and the resulting rho value is 1.43. While ester hydrolysis has long been a favored area for physical-organic investigations of inductive, delocalization, steric, and field effects, the preponderance of the data has been for carbon esters (Jaffé, 1953) rather than phosphorus esters. For the latter, among the best published data available are those of Fukuto and Metcalf (1956) on the hydrolysis of some meta- and para-substituted phenyl diethyl phosphates. In their paper the data are plotted as log k_{obsd} *vs.* sigma. Arrangement of their para-substituted data in a conventional Hammett plot is shown in Figure 2. The resulting rho value is ca. 1.9. This falls within the range listed by Jaffé (1953)

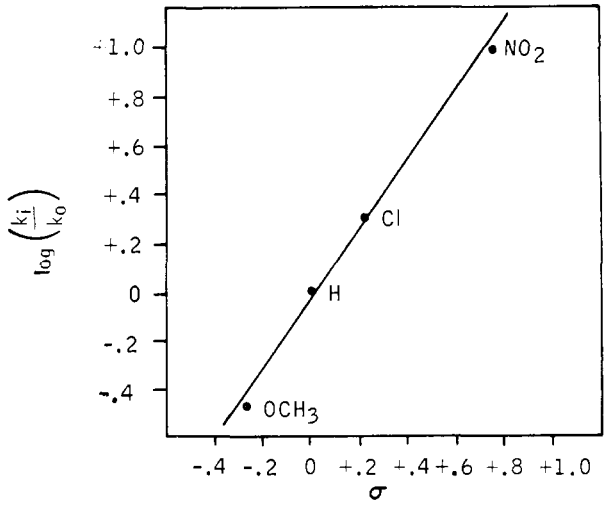

Figure 1. Alkaline hydrolysis of *p*-nitrophenyl esters of 4-substituted phenacyl methylphosphonic acids

In 5.0% *p*-dioxane–95.0% 0.1*M* tris buffer, pH 7.61 at 25° C. *ρ* value (slope), 1.43

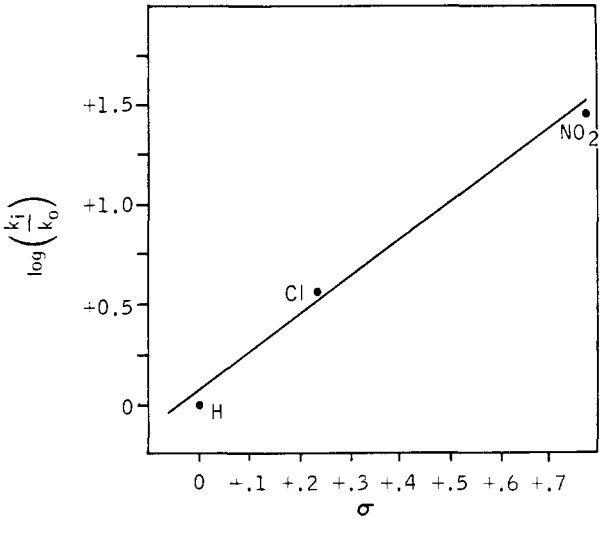

Figure 2. Alkaline hydrolysis of some para-substituted phenyl diethyl phosphates

In 0.1*M* diethyl barbituric acid, pH 9.5, at 37.1° C.
ρ value (slope), ca. 1.9
Data from Fukuto and Metcalf (1956)

for the alkaline hydrolysis of esters of meta- and para-substituted benzoic acids (1.82 to 2.85). While the positive rho values in both our work and that of Fukuto and Metcalf (1956) suggest that electron-attracting substituents are facilitating the reaction via stabilization of the transition state relative to the reactant, further comparisons and comments are best held in reserve until additional Hammett relationships for phosphate and phosphonate esters are available. Currently in this laboratory, work is in progress with a series of para-substituted phenyl phenacyl methylphosphonates.

The results of biological testing of I, II, III, and IV are presented in Table II. A correlation with the hydrolysis data presented in Table I is apparent–that is, the more susceptible the compound is to alkaline hydrolysis, the less toxic. This is to be expected in view of the instability of these compounds in the physiological pH range. They are probably hydrolyzed considerably before reaching vital centers (Levy, 1966). A plot of the approximate LD_{50} values for I, II, III, and IV *vs.* Hammett sigma constants suggests a linear relationship (Figure 3). While the slope (calculated by least squares) is of no special significance, the plot should permit future investigators with this series of compounds to make adequate judgments on the caution to be exercised with any member having a sigma value between −0.3 and +0.8.

EXPERIMENTAL

Synthetic. *p*-NITROPHENYL PHENACYL METHYLPHOSPHONATE (*I*). Phenacyl alcohol (3.264 grams, 0.024 mole) dissolved in 75 ml. of dry benzene was added dropwise over a 30-minute period to a magnetically stirred solution of *p*-nitrophenyl methylphosphonochloridate (de Roos, 1959)

Table II. Acute, Approximate, Intravenous LD_{50} Values of I, II, III, and IV for Mice

(Values in mg./kg. with 95% confidence limits in parentheses)

Compound		
I	14.1 (7.08–28.2) October 1966	14.1 (10.0–20.0) March 1968
II	2.00 (1.26–3.16) October 1966	. . .
III	14.1 (7.08–28.2) October 1966	15.8 (12.6–20.0) October 1966
IV	50.1 (39.8–63.1) October 1966	56.2 (44.7–70.8) March 1968

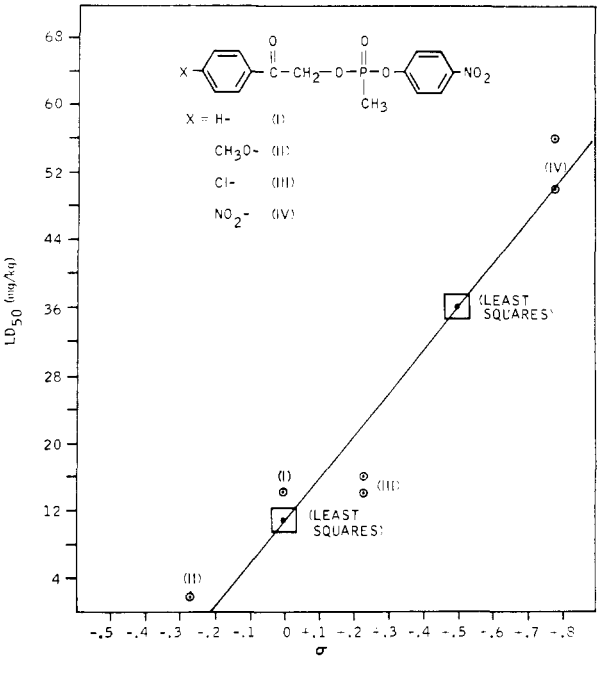

Figure 3. LD_{50} values *vs.* Hammett σ values for I, II, III, and IV

(11.280 grams, 0.048 mole) and dry pyridine (7.6 grams, 0.096 mole) in 50 ml. of dry benzene. An ice bath was used to cool the reaction mixture during the addition. Upon completion of the addition, the stirred reaction mixture was maintained at room temperature for 96 hours. The undissolved fraction (pyridine hydrochloride) was removed by filtration, 50 ml. of dry benzene was added to the filtrate, and the solution was washed progressively with four 50-ml. aliquots of 5% aqueous hydrochloric acid and four 50-ml. portions of 0.1*M* phosphate buffer of pH 7.0. After the solution was dried over Linde Molecular Sieves, Type 4A, the benzene was removed in vacuo to give 4.88 grams (67%) of crude product. Recrystallization from acetone-cyclohexane gave a white crystalline solid (m.p. 112–14° C.). Thin-layer chromatography on silicic acid with 5% acetone–95% chloroform (v./v.) gave one spot (R_f 0.5).

Analysis. Calculated for $C_{15}H_{14}NO_6P$: C, 53.74; H, 5.21; N, 4.17; P, 9.24. Found: C, 53.5; H, 5.1; N, 4.2; P, 9.58.

Infrared (chloroform, Perkin-Elmer Infracord, Model 137): Major peaks at 5.84 (C=O), 6.56 ($PhNO_2$), 7.42 ($PhNO_2$), 7.98 (P=O), 9.02, and 10.71 microns.

Ultraviolet (Beckman DK-2) in *p*-dioxane: Maximum at 243 mμ, E_{mol} 1.58×10^4; 273 mμ, E_{mol} 1.00×10^4.

NMR ($CDCl_3$, tetramethylsilane in $CDCl_3$ as external standard, Varian A-60): τ-values given to approximate center of mass of each peak: 8.05 (3*H*), doublet, J = 19 c.p.s.; 4.48 (2*H*), multiplet; 1.6 to 2.7 (9*H*), multiplet. The doublet at 8.05 represents $-CH_3$, the multiplet at 4.48 $-CH_2-$, and the multiplet at 1.6 to 2.7 the protons on the two benzene rings.

p-METHOXYPHENACYL *p*-NITROPHENYL METHYLPHOSPHONATE (II). *p*-Methoxybenzoyldiazomethane (Wilds and Meader, 1948) (525 mg., 3.01 mmoles) and *p*-nitrophenyl methylphosphonic acid (616 mg., 2.80 mmoles) were added to 10 ml. of acetonitrile and dissolved without warming by swirling. After several minutes, the solution started to turn brown. Most of the solvent was removed under vacuum and a little petroleum ether was added. The solution was seeded and allowed to stand overnight. The resulting gummy brown solid was triturated twice with acetone–petroleum ether. The resulting solid was recrystallized three times from benzene–petroleum ether and twice from methyl ethyl ketone–petroleum ether to give 480 mg. (47%) of product (m.p. 112–13° C.).

Analysis. Calculated for $C_{16}H_{16}NO_7P$: C, 52.61; H, 4.42; N, 3.82; P, 8.48. Found: C, 52.85; H, 4.54; N, 4.14; P, 8.36.

p-CHLOROPHENACYL *p*-NITROPHENYL METHYLPHOSPHONATE (III). *p*-Chlorobenzoyldiazomethane (Wilds and Meader, 1948) (700 mg., 3.98 mmoles) and *p*-nitrophenyl methylphosphonic acid (800 mg., 3.7 mmoles) were dissolved with warming in 10 ml. of dry acetonitrile. After a few minutes on a steam bath, the solution was allowed to stand overnight at room temperature. The solvent was removed under reduced pressure, and the residue was recrystallized three times from benzene–petroleum ether and twice from methyl ethyl ketone–petroleum ether to yield 700 mg. (51%) of product, (m.p. 123–24.5° C).

Analysis. Calculated for $C_{15}H_{13}ClNO_6P$: C, 48.73; H, 3.54; Cl, 9.59; N, 3.79; P, 8.38. Found: C, 48.87; H, 3.81; Cl, 9.69; N, 3.97; P, 8.56.

p-NITROPHENACYL *p*-NITROPHENYL METHYLPHOSPHONATE (IV). *p*-Nitrobenzoyldiazomethane (Bradley and Schwarzenbach, 1928) (564 mg., 2.96 mmoles) and *p*-nitrophenyl methylphosphonic acid (644 mg., 2.95 mmoles) were placed in a flame-dried flask protected by a drying tube. Four milliliters of freshly distilled acetonitrile was added and solution was effected by gentle warming to initiate nitrogen evolution. The solution was allowed to stand in the dark overnight. The product crystallized from the solution together with red-colored impurities. The mother liquors were pipetted away from the crystals, which were then washed with anhydrous ether. The crystals were dissolved in a minimum amount of warm, dry *p*-dioxane. Two volumes of ethyl ether were added, followed by dry petroleum ether to just short of the cloud point. The crystals that appeared were yellow with a slight orange cast. Two recrystallizations gave 613 mg. (53%) of product as light yellow crystals (m.p. 146.5–47.5° C.). A sample from a similar run was recrystallized three times from toluene–*p*-dioxane–petroleum ether for analysis (m.p. 146.0–47.5° C.).

Analysis. Calculated for $C_{15}H_{13}N_2O_8P$: C, 47.38; H, 3.45; N, 7.37. Found: C, 47.34; H, 3.48; N, 7.33.

Kinetics. MATERIALS. Tris(hydroxymethyl)aminomethane (Tris) and its HCl salt, reagent grade (Sigma Chemical Co.), were used without purification. *p*-Dioxane (Fisher Chemical Co.) was purified according to Vogel (1956) and stored in a dark bottle under a nitrogen atmosphere. The 0.1*M* Tris buffer of pH 7.61 (determined by means of a Beckman Research pH meter, Model 101900) was prepared by dissolving 12.120 grams of Tris · HCl, 2.780 grams of Tris · Base, and 0.908 gram of KCl in distilled water and diluting to 1.00 liter at 25.0° C.

PROCEDURE FOR I, II, AND III. A detailed discussion of the technique involved in following the hydrolysis of I in 5.0% *p*-dioxane–95.0% 0.1*M* Tris buffer (v./v.), pH 7.61, at 25.0° C. is given below. Kinetic investigations with II and III were handled in an analogous manner.

The *p*-nitrophenyl phenacyl methylphosphonate (0.0309 gram, 9.217×10^{-5} mole) was dissolved in purified *p*-dioxane and diluted to 100.0 ml. at 25.0° C. Into two 250-ml. glass-stoppered Erlenmeyer flasks, 95.0029 and 94.9981 grams, respectively, of 0.1*M* Tris buffer (density of buffer at 25.0° C. is 1.001 grams per ml.). The Erlenmeyer flasks were then equilibrated at 25.0° C. in a constant temperature bath.

For each run a 4.96-ml. sample (pipet calibrated using *p*-dioxane at 25.0° C.) of the *p*-dioxane–ester solution was added to the buffer with shaking to ensure rapid mixing. Time zero was taken at the initiation of the addition. Following mixing, a sample was quickly transferred to the spectrophotometer cell and the reaction carried out in the thermostated cell compartment at 25.0° C. The *p*-nitrophenol produced during the hydrolysis was determined by measurement of the absorption of the anion at 400 mμ using the Beckman DK-2 spectrophotometer. The production of *p*-nitrophenol was stoichiometric. In each case a plot of $\log [A_\infty/(A_\infty - A_t)]$ *vs.* time, where A_∞ and A_t represent absorbance at times $t = \infty$ and t, respectively, gave straight lines for a minimum of four half lives—i.e., 93.75% completion. The observed k was calculated as a first-order rate constant from the plot described above. Values of observed k for the runs described are recorded in Table I.

The measured pH values of a solution containing 4.96 ml. of *p*-dioxane plus that volume of 0.1*M* Tris buffer needed to give a volume of 100.0 ml. at 25.0° C. agreed within 0.01 pH unit with that of the parent buffer. In addition, the pH of the reaction solution was frequently checked at time 0 and time ∞ and generally showed no change. When a variation was observed, it was never more than 0.01 pH unit.

PROCEDURE FOR IV. A stock solution of *p*-nitrophenacyl *p*-nitrophenyl methylphosphonate was prepared by dissolving 25.8 mg. in 25.0 ml. of purified *p*-dioxane at 25.0° C. The hydrolysis medium was prepared by diluting 4.96 ml. of *p*-dioxane with 0.1*M* Tris buffer of pH 7.61 to 100.0 ml. at 25.0° C. The spectrophotometer cells were charged with 3.02 ml. of the *p*-dioxane–buffer solution and allowed to equilibrate at 25.0° C. On a Teflon mixing rod (shaped like a six-penny nail) was dispensed 40 μl. of the phosphonate ester–*p*-dioxane stock solution. At time zero, the sample on the Teflon stirring rod was introduced into the sample cell of the spectrophotometer with rapid mixing. By this procedure it is possible to obtain the first point in 5 to 6 seconds. The data obtained were handled as given above. Excellent first-order plots were obtained to greater than four half lives and the production of *p*-nitrophenol was stoichiometric. Values of observed k for three runs were 1.20, 1.15, and 1.19 $\times 10^{-1}$ sec.$^{-1}$ The average value is recorded in Table I.

STABILITY OF THE SODIUM SALT OF *p*-NITROPHENYL METHYLPHOSPHONIC ACID. To a 100.0-ml. volumetric flask were added 4.96 ml. of *p*-dioxane, 2.9 mg. of the sodium salt of *p*-nitrophenyl methylphosphonic acid, and 0.1*M* Tris buffer of pH 7.90 to bring the volume to the mark. The solution was kept in a constant temperature bath at 25.0° C. for 27 hours Aliquots were withdrawn periodically and monitored at 400 mμ. The extent of decomposition during the 27-hour period was ca. 0.25%.

Toxicity Testing. In conducting the research described, the investigators adhered to the policies promulgated by the Committee on the Guide for Laboratory Animal Resources (United States Public Health Service, 1963).

Charles River-Swiss male albino mice, weighing between 18 and 25 grams, were each given a single injection via one of the lateral tail veins, housed individually, and observed for death in the subsequent 24 hours. Prior to injection the mice were allowed free access to food and water. Geometric series of doses were employed with spacing of 0.1 log, except for the October 1966 determinations on I and the October 1968 determinations on III. In these two cases, a spacing of 0.3 log was used. Two mice were injected at each dose level and the data analyzed by the method of Thompson (1947), Thompson and Weil (1952), and Weil (1952). The results are tabulated in Table II.

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