The Relation between Basicity and Selectivity in Organophosphates

R. D. O'BRIEN and B. D. HILTON
Department of Entomology,
Cornell University,
Ithaca, N. Y.

Amiton is more toxic to mammals than to insects. Confirmation was sought for the hypothesis that this adverse selectivity is due to the ionization of amiton, whose pKa is 8.5. Fluoro derivatives of amiton and related compounds were made, with pKa's ranging from 8.9 to 4.2. It was confirmed that adverse selectivity decreased with decreasing basicity. There was strong evidence that only the protonated forms of the P(O)S compounds were potent anticholinesterases. The P(O)O analogs had little anticholinesterase activity.

THERE is wide interest in organophosphates which have high insect toxicity and low mammalian toxicity. In a few cases, the reverse pattern is found; such a pattern is of interest to insecticide chemists in indicating what sort of compounds to avoid. In the case of amiton (*O,O*-diethyl *S*-2-diethylaminoethyl phosphorothioate), this adverse selectivity is attributable to the ionization of the compound, whose pKa is 8.5, and which is consequently 97% protonated at pH 7. The attribution was based on two facts—the rate of penetration of amiton into the cockroach nerve cord showed a pH dependence which implied that only the unprotonated form penetrated the cord (*9*, *10*); and the neuromuscular junction, which in mammals is the vital cholinesterase site available to ionic materials, does not in insects use cholinesterase (*4*). Consequently, ionized anticholinesterases are ineffective against most insects (*6*, *11*).

A corollary of the above argument should be that if one could weaken the basicity of amiton, one should produce a less selective toxicant. This has proved to be the case in the present study.

Experimental

Synthesis. The compounds shown in Table I were prepared by a modification of the method of McIvor (*7*) as follows. Approximately 4 grams of the sodium or potassium salt of diethyl phosphate, *O,O*-diethyl phosphorothioate, or *O,O*-diethyl phosphorodithioate was reacted with one half of a molar equivalent of 2-chlorotriethylamine hydrochloride or the appropriate fluoro derivative by refluxing for 24 hours in acetone. In the case of compounds VII and IX, however, equimolar quantities of the alkyl phosphate salt and the free base form of the amine were used (*7*); later, the above modified method was found to be preferable. The product was cleaned up by partitioning between chloroform and water at various pH's and removing unreacted amine under vacuum. Attempts to isolate oxalate salts of the small quantities available were not successful. Purity was established on the basis of phosphorus content, neutral equivalent, infrared spectrum, and a single component with respect to both phosphorus (*3*) and amine (*1*) in a paper chromatographic system (*15*).

Compounds IV through VII gave rather low values for phosphorus content. The impurities are unknown, but are not compounds which respond on paper chromatograms to the phosphorus or the amine reagents given above.

The fluorinated chlorotriethylamines were made as follows. 2-Fluoroethanol was prepared from potassium fluoride and ethylene chlorohydrin (yield 43%) and brominated with PBr_3 (yield 55%) to give FC_2H_4Br (*13*). For the monofluoroamine, 2-ethylaminoethanol was stirred and refluxed with a 25% excess of FC_2H_4Br in anhydrous benzene over K_2CO_3 for 40 hours giving 2-fluoro-2′-hydroxytriethylamine. The yield was 75%. Treatment of this with a 60% excess of thionyl produced a 77% yield of the hydrochloride of the desired amine, 2-chloro-2′-fluorotriethylamine (*7*). For the difluoroamine, the fluoroethyl bromide was treated with anhydrous ammonia in ethanol, by heating in a bomb at 75° C. for 7 hours, to give a 26% yield of bis(2-fluoroethyl)amine. This was treated with ethylene oxide in the cold, and the resultant 2-hydroxyethyl-bis(2-fluoroethyl)amine was converted, without isolation, to the hydrochloride, and treated with thionyl chloride, producing the hydrochloride of 2-chloroethyl-bis(2-fluoroethyl)amine (*2*). This was recrystallized in ethyl acetate in 55% yield.

pKa Determination. The pKa was determined by microtitration with concurrent pH determination; the pKa given is the inflection point of the plot of pH against added acid.

Cholinesterase Inhibition. The acetylcholinesterase source was human red cell ghosts, whose preparation and inhibitory assay were described before (*8*.

Toxicity. Female white mice (Rolfsmeyer Farms, Madison, Wis.) were used. The compounds were dissolved in phosphate buffer of pH 7.4, 0.1*M*, and injected intraperitoneally. The houseflies were a Chemical Specialities Manufacturer's Association (CSMA) susceptible strain. The 3- or 4-day-old adult females were treated topically on the dorsal thorax with 1 μl. each of an acetone solution. Observations on mice and flies were made after 24 hours.

Results

pKa. Table I shows that the pKa's were lowered by fluorination, as one would anticipate. One fluorine lowered the pKa by 2.2 units for the P(O)O compounds, 2.0 units for the P(O)S, and 2.0 units for the P(S)S. The corresponding figures for two fluorines were 3.9, 3.8, and 4.0. These are profound changes. Since the pKa of methylamine is lowered only 3.1 units by substitution with two fluorines (*5*), it is probable that one must invoke field and shielding effects as well as the inductive effect (which will be small, because of the 2-carbon separation of F and N) to account for the changes observed.

It had been anticipated that P(O) compounds would be weaker bases than P(S) compounds, since there is ample evidence (*10*) of the greater electrophilic effect of P(O) than P(S). But the reverse was observed—in the unfluorinated series, substituting P(S)S for P(O)S lowered the pKa 0.3 unit, in the monofluoro series 0.3 unit, and in the difluoro series 0.5 unit. A possible explanation is that the preferred orientation of the molecules involves proximity of the (O) or (S) to the nitrogen; the (S), being larger, would shield move. Stuart-Briegleb models showed that the shielding of the nitrogen is quite effective in such circumstances.

The thiolo sulfur was also base-weakening compared to oxygen. Substituting P(O)S for P(O)O reduced the pKa 0.4 unit in the unfluorinated, 0.2 unit in the monofluorinated, and 0.3 unit in the difluorinated series. The effect may be due to shielding plus a small contribution by the greater inductomeric polarizability of the sulfur.

Table I. Basicities and Analyses

No.	Compound	pKa[a]	Neutral Equivalent % Calcd.	Neutral Equivalent % Found	Phosphorus Content % Calcd.	Phosphorus Content % Found	R_f	% Yield
I	$(C_2H_5O)_2P(O)OC_2H_4N(C_2H_5)_2$	8.9	5.53	5.81	12.2	12.0	0.76	67.8
II	$(C_2H_5O)_2P(O)SC_2H_4N(C_2H_5)_2$ Amiton	8.5	5.20	5.67	11.5	11.4	0.74	..
III	$(C_2H_5O)_2P(S)SC_2H_4N(C_2H_5)_2$	8.2	4.91	5.25	10.9	10.8	0.82	81.6
IV	$(C_2H_5O)_2P(O)OC_2H_4N(C_2H_4F)(C_2H_5)$	6.7	5.16	4.91	11.4	10.7	0.77	79.9
V	$(C_2H_5O)_2P(O)SC_2H_4N(C_2H_4F)(C_2H_5)$	6.5	4.88	4.78	10.8	9.76	0.75	70.1
VI	$(C_2H_5O)_2P(S)SC_2H_4N(C_2H_4F)(C_2H_5)$	6.2	4.62	4.34	10.2	9.55	0.87	75.4
VII	$(C_2H_5O)_2P(O)OC_2H_4N(C_2H_4F)_2$	5.0	4.84	5.09	10.7	9.89	0.90	60.7
VIII	$(C_2H_5O)_2P(O)SC_2H_4N(C_2H_4F)_2$	4.7	4.59	4.77	10.1	9.98	0.93	62.5
IX	$(C_2H_5O)_2P(S)SC_2H_4N(C_2H_4F)_2$	4.2	4.36	4.53	9.64	9.49	0.98	68.8

[a] The pKa's were obtained in water and are not corrected for activity. Agreement between replicates was within 0.1 unit. Paper chromatography was carried out in the system of Tammelin (*15*), and phosphorus (*3*) and amines (*1*) were detected separately. Agreement between R_f replicates was within 0.02 unit. Replicates of measurements of neutral equivalents and phosphorus determinations agreed within 2% of the indicated values.

Table II. Toxicities and Anticholinesterase Activities

No.[a]	Abbreviated Name	pI_{50}, Red Cell	Mouse LD_{50} (M)	Fly LD_{50} (F)	Selectivity, F/M
I	P(O)OHH	<3	>100	535	<5.4
II	P(O)SHH (Amiton)	8.1	0.3	100	330
III	P(S)SHH	...	2.5	70	28
IV	P(O)OHF	<3	>100	96	<0.96
V	P(O)SHF	7.0	0.7	35	50
VI	P(S)SHF	<3	2.5	52	21
VII	P(O)OFF	<3	75	22	0.29
VIII	P(O)SFF	5.2	1.5	19	13
IX	P(S)SFF	...	3.0	24	8.0

[a] The numbers refer to the same compounds given in Table I. pI_{50} values are negative logarithms of concentration inhibiting red cell cholinesterase by 50%. LD_{50} values in mg./kg. body weight.

Anticholinesterase Activity. It was expected that the three P(S) compounds would be without anticholinesterase activity, as is almost invariably the case except with a few well-defined exceptions (*10*). Of the P(S) compounds, only the monofluoro derivative (VI) was assayed against cholinesterase, and it was indeed ineffective (Table II).

The P(O)S compounds (II, V, VIII) were all effective inhibitors of red cell cholinesterase (Table II). The effectiveness decreased with decreasing basicity, the pI_{50}'s falling off in the order 8.1, 7.0, 5.2. This conforms with the evidence (*9, 12*) that the protonated form of such inhibitors is a much better inhibitor than the free base. At the pH of assay, which was 7.4, the percentage protonation of the three compounds would be 93, 11, and 0.2, respectively. If one assumes that only the protonated forms are active, the corrected pI_{50}'s for these compounds may be calculated as 8.13, 7.96, and 7.90, respectively. (Sample calculation: VIII is 0.2% or 1/500 ionized, thus pI_{50} should be increased by log 500 = 2.7). This remarkable agreement strongly suggests that the assumption is correct.

Quite unexpectedly, the P(O)O compounds were all very poor anticholinesterases, with pI_{50}'s of less than 3 for the red cell enzyme.

Toxicity. The toxicity of the P(O)O compounds to mice or flies was usually very low, as one would expect from such poor anticholinesterases (Table II). An exception was VII, the P(O)OFF, which was toxic to flies, with an LD_{50} of 22 mg. per kg. Could this effect be due to the fact that the compound was a good inhibitor specifically of fly cholinesterase? The pI_{50} against fly-head cholinesterase was measured, and found to be 4.2. Therefore the compound is a poor inhibitor, even though it is over 16 times better than against the red cell enzyme and is more effective against fly cholinesterase than its unfluorinated analog (I) whose pI_{50} was less than 3. The P(O)OFF compound had a small inhibitory effect in vivo—a dose of 75 mg. per kg. to flies gave 37% inhibition of whole-body cholinesterase after 2 hours.

Discussion

The hypothesis that basicity governs selectivity in this class of compounds is confirmed. A previous recommendation (*9*) was that in designing insecticides, "...it is desirable to avoid ionizable compounds, unless the pKa is low in the case of bases—e.g., below 7—..." In the amiton derivatives, decreasing the basicity decreases the adverse selectivity (Table II). The selectivities of the unsubstituted, monofluoro, and difluoro compounds were for the P(O)S series 330, 50, and 13, respectively; and for the P(S)S series 28, 21, and 8. The P(O)O series cannot be specifically evaluated, the data being <5.4, <0.96, and 0.29, which suggests the same trend.

The effect of the thiono [P(S)] sulfur is to reduce the adverse selectivity—e.g., in the HH series—from 330 to 28; in the HF series, from 50 to 21; in the FF series, from 13 to 8. Favorable selectivity—i.e., greater toxicity to insects than to mammals—is often due to metabolic rather than (as in the present case) to penetrative factors, in which case the thiono sulfur increases favorable selectivity, probably due to the "op-

portunity factor" by which extra time is given for degradation to occur, because of the time lag conferred by the need for bioconversion of the P(S) to P(O) (*10*). In this case, the selectivity is always such as to spare the mammal. Amiton is known to be degraded in liver (*14*). If this degradation was more vigorous in mammals than in insects, the thiono sulfur would have the effect of raising the mammalian LD_{50} by this same "opportunity factor," and so reducing adverse selectivity.

A paradox still unresolved is: if adverse selectivity in these compounds is due to failure of the protonated material to penetrate the nerve cord, why is (for example) the P(O)SFF still 13-fold selective, even though it has a pKa of 4.7, so that at pH 7 only 0.5% would be protonated? The effect is not attributable to poor lipid solubility of the free base, for its partition ceofficient, water: olive oil, was found to be 1.06. It could be caused by target selectivity—i.e., the cholinesterase of the housefly being more sensitive than that of the mouse. Alternatively, it may be connected with the fact that the housefly applications were topical, so that an extra barrier (the cuticle) was interposed in this case.

Certain compounds which were poor anticholinesterases in vitro were nevertheless toxic to flies or mice. In the case of P(S) compounds, the reason is undoubtedly that these are latent inhibitors, and are activated to P(O) derivatives in vivo. In one case, the P(O)OFF compound, toxicity to houseflies was encountered in spite of poor inhibition of fly cholinesterase in vitro (pI_{50}, 4.2), and no precedent exists for activation of such a compound to a more potent anticholinesterase. Suspicion that the fluorinated compounds might kill by some effect other than cholinesterase inhibition,—e.g., by forming fluoracetate from a fluoroethyl group—was lessened when it was found that one of the "half-molecules," (ethyl-2-fluorethyl)-2-aminoethanol, had little toxicity to mice, for 200 mg. per kg. (intraperitoneal) was not lethal.

Acknowledgment

The authors are grateful to Elaine Dannelley for her expert technical assistance. This work was supported in part by grants from the Cooperative G.L.F. Exchange, Inc., and project RG 7804 of the National Institutes of Health.

Literature Cited

(1) Block, R. J., Durrum, E. L., Zweig, F., "A Manual of Paper Chromatography and Paper Electrophoresis," p. 351–3, Academic Press, New York, 1958.
(2) Childs, A. F., Goldsworthy, L. J., Harding, G. F., King, F. E., Nineham, A. W., Norris, W. L., Plant, S. G. P., Selton, B., Tompsett, A. L. L., *J. Chem. Soc.* **1948,** p. 2174.
(3) Hanes, C. S., Isherwood, R. A., *Nature* **164,** 1107 (1949).
(4) Harlow, P. A., *Ann. Appl. Biol.* **46,** 55 (1958).
(5) "International Critical Tables," Vol. **6,** p. 259, McGraw-Hill, New York, 1929.
(6) Kolbezen, M. J., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **2,** 864 (1954).
(7) McIvor, R. A., Defense Research Chemical Laboratories, Ottawa, Canada, private communication, 1960.
(8) O'Brien, R. D., J. AGR. FOOD CHEM. **11,** 163 (1963).
(9) O'Brien, R. D., *J. Econ. Entomol.* **52,** 812 (1959).
(10) O'Brien, R. D., "Toxic Phosphorus Esters," Academic Press, New York, 1960.
(11) O'Brien, R. D., Fisher, R. W., *J. Econ. Entomol.* **51,** 169 (1958).
(12) Ooms, A. J. J., Ph.D. thesis, Leiden University, Holland, 1961.
(13) Saunders, B. C., Stacey, G. J., Wilding, I. G. E., *J. Chem. Soc.* **1949,** p. 773.
(14) Scaife, J. R., Campbell, D. H., *Can. J. Biochem. Physiol.* **37,** 279 (1959).
(15) Tammelin, L. E., *Acta Chem. Scand.* **11,** 1738 (1957).

Received for review October 15, 1962. Accepted March 7, 1963.

INSECTICIDES FROM PLANTS

Nicandrenone, A New Compound with Insecticidal Properties, Isolated from *Nicandra physalodes*

OLGA NALBANDOV, ROBERT T. YAMAMOTO,[1] and GOTTFRIED S. FRAENKEL

Department of Entomology, University of Illinois, Urbana, Ill.

An insect repellent substance with toxic properties has been isolated in chromatographically pure form from leaves of the plant *Nicandra physalodes*. The molecular formula $C_{34}H_{42}O_7$ has been established for the compound, and evidence is presented which indicates that it is a conjugated ketone. The name "nicandrenone" is suggested. Initial studies aimed at elucidation of the structure of the compound are reported; these include infrared, ultraviolet, and nuclear magnetic spectra.

IN NATURE, the tobacco hornworm feeds on only a few plants, all of which are members of the family *Solanaceae*. Laboratory feeding tests in which fresh foliage was used showed that many other plants of this family are palatable to the insect, although some which were readily eaten produced toxic effects (*13*). In the same experiment, a few solanaceous plants were found to be repellent with little or no feeding occurring on them. A plant in the latter category is *Nicandra physalodes*. The repellent factor can be extracted from the leaves of this plant with water and removed from the aqueous solution by extraction with chloroform or ether. Removal of solvent from the latter extracts leaves solid material which has been found to be highly toxic to house flies as well as to the hornworm (*2*). Fractionation of this crude material and investigations into the chemical composition of the purified toxic substance are reported in this study.

This material has been isolated by other investigators (*4*), but its repellent and toxic properties toward insects were not observed. The name "nicandrin" was proposed for what was probably an impure preparation. The conclusion was reached that nicandrin is a glycoside.

In searches for naturally occurring insecticides, previous investigations failed to demonstrate toxic activity in the plant *Nicandra physalodes* (*6*). Houseflies were used as test insects in one study in which petroleum ether, ethyl ether, and chloroform extracts of the whole plant were found to be nontoxic. Another investi-

[1] Present address: Entomology Research Division, U.S. Department of Agriculture, Agricultural Research Center, Beltsville, Md.