Effect of a Thiono Substituent on Toxicity of Fluorophosphates to Insects and Mice

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Thiono analogs were prepared of diisopropyl phosphorofluoridate (DFP), N,N'-diisopropyl-phosphorodiamidic fluoride (mipafox), and tetramethylphosphorodiamidic fluoride (dimefox). The toxicities for houseflies, bees, and mice were compared for the P(O) and P(S) analogs. In most cases the P(S) was somewhat less toxic to mice and insects than the P(O) analog. The P(S) showed a little more adverse selectivity toward insects as compared with mice than did the P(O) analog for the cases of DFP and dimefox, but the opposite was found for mipafox. All such effects were small, typically two- or threefold. The implications for the concept of an "opportunity factor" are discussed.

IN AN attempt to establish principles for the design of organophosphates of predictable toxicity, the role of groups susceptible to degradation and activation has been studied (*12, 14*). Many organophosphates which are rapidly degraded by a given species have low toxicity to that species; rates of degradation vary considerably between species, and such differing rates can be an important factor in causing selective toxicity—i.e., differences in toxicity to different organisms. Phosphorothionates are usually more selectively toxic to insects, as compared with mammals, than their phosphate analogs (*13, 14*). This selectivity has been attributed to the fact that most phosphorothionates require conversion ("activation") to the phosphate which is usually the actual toxicant, and the consequent delay in intoxication gives a greater opportunity for differences in degrading enzymes to be effective. Thus the conditions for this "opportunity factor" to be demonstrable are the presence in the compound of a group which can be degraded faster in one organism than another, and a compound which requires activation.

It was felt that it would be a dramatic demonstration of the concept of an opportunity factor if one could show that the mammalian toxicity of a compound notorious for its mammalian toxicity was greatly reduced in the case of its thiono analog. The warfare agent diisopropyl phosphorofluoridate (DFP) is very toxic to insects and mammals (*7*), yet in mammals there is an active degrading enzyme, "DFP-ase," present in numerous tissues (*10*). The thiono analog ought to have lessened toxicity to mammals, and if the degrading enzymes were less effective in insects than mammals (as is commonly found with organophosphates) one might achieve a selectively toxic compound,

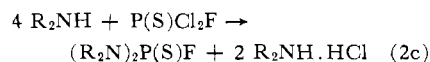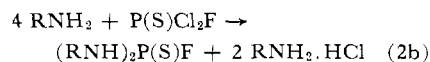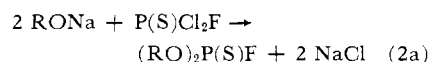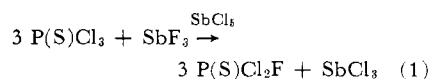and thus adapt a warfare agent for use as an insecticide.

To extend the generality of the observations, thiono analogs of the other well known insecticidal fluoridic organophosphates, tetramethylphosphorodiamidic fluoride (dimefox) and N,N'-diisopropylphosphorodiamidic fluoride (mipafox), were prepared. The metabolism of dimefox has been extensively studied (*1*), but metabolism of mipafox has not been studied in animals.

The biological properties of the thiono compounds described here have not been hitherto reported. Nine dialkyl phosphorothionic fluorides (not including thiono-DFP) were stated in 1957 to be contact insecticides of low toxicity for warm-blooded animals, but no data were given (*4*).

Methods

Mipafox and dimefox were obtained from L. Light and Co., Colnbrook, England, and DFP from Sigma Chemical Co., St. Louis, Mo.

The thiono analogs were prepared according to the scheme of Olàh *et al.* (*16, 17*), who prepared a number of phosphorothionic fluorides, including thiono-DFP but not thiono-mipafox or thiono-dimefox.

$$3 \ P(S)Cl_3 + SbF_3 \xrightarrow{SbCl_5}$$
$$3 \ P(S)Cl_2F + SbCl_3 \quad (1)$$

$$2 \ RONa + P(S)Cl_2F \rightarrow$$
$$(RO)_2P(S)F + 2 \ NaCl \quad (2a)$$

$$4 \ RNH_2 + P(S)Cl_2F \rightarrow$$
$$(RNH)_2P(S)F + 2 \ RNH_2.HCl \quad (2b)$$

$$4 \ R_2NH + P(S)Cl_2F \rightarrow$$
$$(R_2N)_2P(S)F + 2 \ R_2NH.HCl \quad (2c)$$

Reactions 2a, 2b, and 2c appear to be perfectly straightforward, giving yields ranging from 70 to 85% of the desired analogs. The following preparation of

N,N' - diisopropylphosphorodiamidothionic fluoride (thiono-mipafox) demonstrates a typical synthesis.

A 250-ml. three-necked flask, fitted with a condenser and drying tube, mechanical stirrer, thermometer, dropping funnel, and nitrogen inlet, was charged with a mixture of 25 ml. (0.292 mole) of isopropylamine in 50 ml. of anhydrous benzene. The system was flushed thoroughly with nitrogen. Stirring was started, and the flow of nitrogen increased to maintain a positive pressure of nitrogen while the flask and its contents were cooled to −5° C. When equilibrium was reached, the nitrogen was stopped, and a solution of 10 grams (0.066 mole) of $P(S)Cl_2F$ in 50 ml. of anhydrous benzene was added at a rate of one drop every 2 seconds. After the addition, the bath was removed, and the reaction stirred an additional 2 hours at room temperature. The mixture was allowed to stand overnight and then filtered through a medium-grade sintered-glass funnel. The amine salt was broken up, stirred vigorously in fresh benzene, and refiltered. Evaporation of the combined filtrates at water pump pressure gave a viscous liquid residue which crystallized readily when dry ice was touched to the side of the flask.

The crude thiono-mipafox could be recrystallized from many of the hydrocarbon solvents. With *n*-heptane, colorless flat prisms were obtained, some of which measured 1 cm. in length, melting at 46.2–47.4° C. Sublimation of these crystals did not appear to change the melting point. The yield was 11.0 grams (85%). Analysis showed, in per cent: C calcd. 36.35, found 36.26; H calcd. 8.14, found 8.18; F calcd. 9.58, found 9.65.

Tetramethylphosphorodiamidothionic fluoride (thiono-dimefox) prepared as in Reaction 2c, was a colorless oil boiling at 61.9–62.0° C. (1 mm.), n_D^{23} 1.4734. It fumed readily on contact with moist air. Analyses showed, in per cent:

Table I. Toxicity of Fluorophosphates

| | LD₅₀, Mg./Kg. | | | Selectivity Factor | |
	Mouse	Housefly	Bee	Housefly/ mouse	Bee/mouse
DFP	8.5	35	>100	0.24	<0.09
Thiono-DFP	7.1	>100	>100	<0.07	<0.07
Dimefox	1.35	19	>100	0.07	<0.01
Thiono-dimefox	3	>100	>100	<0.03	<0.03
Mipafox	15.5	10	40	1.6	0.39
Thiono-mipafox	58	21	46	2.8	1.3

Mouse data are for intraperitoneal, insect data for topical route. Selectivity factor is calculated—e.g., for bee/mouse from LD_{50} mouse/LD_{50} bee, since LD_{50} is inverse measure of toxicity.

C calcd. 28.29, found 28.50; H calcd. 7.11, found 7.31; F calcd. 11.16, found 11.38.

Toxicity at 24 hours was determined on approximately 20-gram female white mice (Rolfsmeyer Co., Madison, Wis.) by intraperitoneal injection of solutions in propylene glycol; and on worker bees (Cornell colony) and 3-day old female houseflies (Wilson strain) by topical application of solutions in acetone, except for mipafox which required solution in methanol.

Results and Discussion

In the following discussion, all data are expressed as milligrams per kilogram.

The toxicity of the compounds to insects and mice is shown in Table I. It was expected that the replacement of (O) by (S) would decrease the toxicity of all three P(O) compounds for all organisms; and it was hoped that the decrease might be greater for a mammal than for an insect, because of the greater efficiency of mammalian than of insect degrading systems. Thus the P(S) compounds should be more selectively toxic to insects than their P(O) analogs.

Comparisons with Literature. For dimefox, intraperitoneal LD_{50}'s for mice of 1.4 (15), 1.2 (5), and 5.0 (3) have been reported, in good agreement with our value of 1.35. An oral LD_{50} of 191 has been reported for bees (6), in good agreement with our value of >100 for topical LD_{50}. For mipafox, no mouse intraperitoneal data are available; LD_{50}'s for various mammals by various routes range from 25 to 100 (11). A topical LD_{50} for mipafox to houseflies of 7 has been reported (7), in good agreement with our value of 10. For bees, only an oral LD_{50} for mipafox has been reported (11): It is 14, which is roughly compatible with our topical LD_{50} of 40, in view of the difference in routes. Our values for DFP are in poorer agreement with the literature, which suggests topical LD_{50}'s of 30 for bee (9); of 15 (9) and 12 (7) for housefly; and 4 for mouse (5); unlike our values of >100, 35, and 8.5, respectively. As a check on

the purity of the DFP, an infrared spectrum was prepared which corresponded well with a published spectrum (8). Excellent results from elemental analyses were then obtained, as follows (in per cent): C calcd. 39.14, found 39.05; H calcd. 7.66, found 7.36; F calcd. 10.32, found 10.18. Such characterization of DFP was not provided in the above references, which reported toxicity results differing from ours.

Data of Table I. The first unexpected feature was the insensitivity of the bee to DFP and dimefox. Krueger and Casida (7) found that for five of six insect species studied, DFP and mipafox were very toxic; an exception was the black carpet beetle, *Attagenus piceus*, for which topical LD_{50}'s of >1000 for DFP and 630 for mipafox were found. One might have attributed this insensitivity to poor penetration through the very thick cuticle of this insect. However, the findings with the bee suggest that such insect insensitivity may be due to other factors, such as abnormal cholinesterase [which seems to be a major factor in the insensitivity of bees as compared with flies to diisopropyl parathion and diisopropyl paraoxon (2)] or rapid degradation.

In comparing the P(O) with the corresponding P(S) compounds, it is apparent that in six of the seven cases for which comparisons were possible, the P(S) analog was the less toxic. However, the differences were not very large, being >5 only for one case: dimefox for the housefly. A two- or threefold decrease was more typical. In a single case (DFP for mouse) the P(S) analog was a trifle more toxic, 1.2-fold, than the P(O).

The selective toxicity of the parent P(O) compounds toward insects as compared with mice was adverse for DFP and dimefox—i.e., the compounds were more toxic to the mice. Mipafox showed a little desirable selectivity for housefly (1.6 × more toxic than to mice) but adverse for the bee (LD_{50} 0.39 × the value for mice). The effect of substituting P(S) for P(O) was measurable in only four cases. For DFP and dime-

fox, the effect was to make the selectivity even more adverse, by a factor of two- or threefold. But with mipafox a small improvement in selectivity was found: 1.7-fold for the housefly and 3.3-fold for the bee.

It is apparent that in this class of compounds, very little is gained by substituting P(S) for P(O). This is in contrast to the claim (13) that "It is almost invariably true that P(S) compounds are less toxic to mammals and to insects than their P(O) analogs; and are more selectively toxic to insects as compared to mammals." The claim was supported by a tabulation comparing the toxicity of P(O) analogs with those of four P(S) insecticides: dimethoate, malathion, acethion, and parathion. The contrasting findings recall the test of the opportunity factor argument conducted by Spencer (19). He compared P(S) and P(O) analogs of the *cis* and *trans* isomers of mevinphos (dimethyl 1-methyl-2-carboxyethylvinyl phosphate), and in comparing mice and houseflies found that the P(S) analog was a little less selectively toxic to houseflies (1.4-fold) than the P(O), in the case of the *cis* isomer, but more selective (65-fold) in the case of the *trans*. It was suggested that this difference reflected stereospecificity in the degrading enzymes presumed to be responsible for the differential toxicity.

Let us consider the implications which the present failure to produce the anticipated toxic properties by introducing a thiono sulfur has for the concept of an opportunity factor. One possibility is that the concept is wrong—for instance, the difference in P(S) and P(O) selectivity often observed might be due to the fact that in some cases there are degrading enzymes effective against the P(S) but not against the P(O) analog, and that these enzymes differ considerably between organisms. On the other hand, the opportunity factor concept may not be operative for the present compounds, either because activation via the P(S) group is not required, or because such activation is so rapid that it is not a limiting factor. Activation of dimefox probably occurs by hydroxylation of a methyl group (1), but it had been hoped that additional activation via the P(S) would be required for thiono-dimefox. Finally, in our experiments on insect toxicity (but not on mouse toxicity) topical application was used, so that a permeability factor could be operative. The P(S) analogs may have shown less insect toxicity in part because apolarity worsens penetration of compounds applied in an organic solvent to insect cuticle (18).

Acknowledgment

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INSECTICIDE SCREENING

Synthesis and Insecticide Activity of Methyl 2,4,5-Trichlorophenyl Phosphoroamidates

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The synthesis, physical properties, and insect toxicities of a series of methyl 2,4,5-trichlorophenyl phosphoramidates are described. The amidophosphates were prepared by the controlled methanolysis and aminolysis of 2,4,5-trichlorophenyl phosphorodichloridate. While the amidophosphates show a wide spectrum of insecticidal activity, practical use against agricultural pests is limited by phytotoxicity to host plants and short residual toxicities to insects on treated foliage, soil, or aqueous habitats.

THE synthesis, physical properties, and insect toxicities of a series of O-methyl O-(2,4,5-trichlorophenyl) phosphoramidothioates were recently reported (2). As a continuing part of this study we have prepared the corresponding phosphoramidates in high purity for comparative insecticidal evaluation,
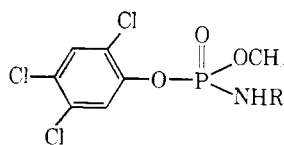
Chemical Studies

2,4,5-Trichlorophenyl phosphorodichloridate appeared to be an ideal starting material, since many phosphorus acid chlorides of this type are readily prepared by heating a phenol with excess phosphoryl chloride in the presence of a metal halide catalyst (8, 10). The stepwise esterification and amidation of this dichloridate under controlled conditions would be expected to give the desired methyl 2,4,5-trichlorophenyl phosphoramidates.

I

II

III
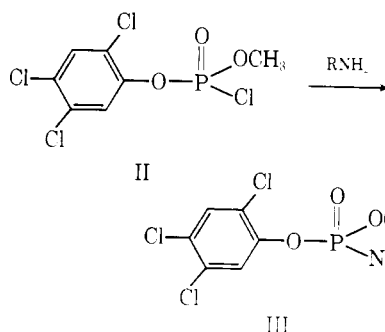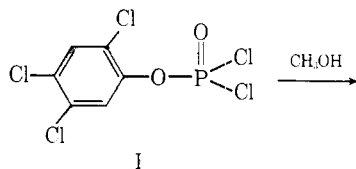
We had difficulty, however, in preparing the 2,4,5-trichlorophenyl phosphorodichloridate using magnesium chloride or magnesium metal as a catalyst. Yields ranged from 0 to 45%. These results are in agreement with those of Orloff, Worrel, and Markley (8), who found that the yield of dichloridate was decreased by the presence of electron-withdrawing substituents in the aryl group. We have noted that traces of catalyst in the crude product appear to promote disproportionation at distillation temperatures.

An improved method for the preparation of 2,4,5-trichlorophenyl phosphorodichloridate was to add molten 2,4,5-trichlorophenol to an excess of phosphoryl chloride containing pyridine equimolar to the amount of phenol. The pyridine hydrochloride was filtered

and the product distilled under reduced pressure.

Very little information outside the patent literature (1, 5, 9) is available on the esterification and amidation of aryl phosphorodichloridates. Orloff, Worrel, and Markley (8) prepared triesters by the solvolytic reaction of aryl phosphorodichloridates with alcohols.

We have prepared triesters in a similar manner. Dimethyl 2,4,5-trichlorophenyl phosphate (IV) was prepared in high yields by treating I with excess methanol.