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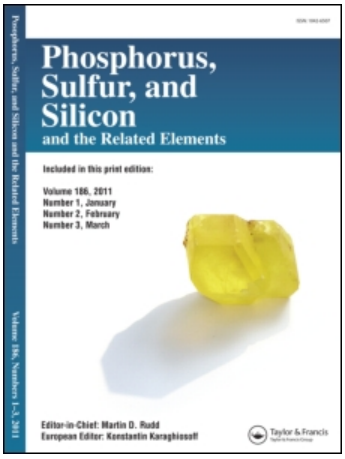

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THIONO COMPOUNDS. 8. MUTAGENIC ACTIVITY OF REPRESENTATIVE AMIDES OF THIOPHOSPHORIC ACID

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THIONO COMPOUNDS. 8. MUTAGENIC ACTIVITY OF REPRESENTATIVE AMIDES OF THIOPHOSPHORIC ACID[1]

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Amides of thiophosphoric acid were studied, in order to initiate correlations of mutagenicity with structure, by use of a modified Ames assay. Representative thiophosphoramides of the structure $(XArNH)_3PS$ were not mutagenic, irrespective of whether X was a reference hydrogen atom, an electron-withdrawing, or an electron-donating group. One phosphoramidothioate of the structure $(2,4\text{-}X_2ArNH)P(S)(OCH_2CH_3)_2$ effected base-pair mutation when X was F (but not when X was CH_3), when S-9 liver homogenate with exogenous NADP was used; when X was H, only inconsistent mutagenic activity following metabolic activation was observed even at concentrations near those that produced acute cellular toxicity. Mutagenicity of these N-arylamides thus appears to follow guidelines concluded for esters, $(RO)_3PS$, i.e. that mutagenesis is most probable when two groups are small enough to permit nucleophilic attack by a biomacromolecule on the electrophilic phosphorus atom. That the third group should be electron withdrawing again seems important although it need not be a good leaving group. An alkyl thionamide, $[(CH_3)_2N]_3PS$, resembled the oxygen counterpart, $[(CH_3)_2N]_3PO$, to which it probably is biotransformed, in being only marginally mutagenic. We conclude that the hazard of mutagenesis is likely to be less with amides than with esters of thiophosphoric acid and that most of the representative thioamides tested are unlikely to pose serious mutagenic hazards.

Key Words: Ames assay, base-pair mutation, mutagenesis, phosphoramidothioate, thionamide, thiophosphoramide

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INTRODUCTION

Thiono compounds, i.e. those that contain a doubly bonded sulfur atom, include esters of thiophosphoric acid of structure I (phosphorothioates), amides of structure II (thiophosphoramides) and amido esters of structure III (phosphoramidothioates) (Figure 1). In view of the widespread use of such compounds, initiation of a study of structure-activity relations was desirable that would contribute to an ultimate capability for predicting the types of groups most likely to lead to hazards of mutagenicity or carcinogenicity when such groups are used as R^1–R^{11} in structures of types I–III. We reported previously on some typical compounds of structure I and concluded that "The mutagenic phosphorothioates contained a strong electron-withdrawing and/or a good leaving group, together with two other groups small enough to permit nucleophilic attack by a biomacromolecule on the electrophilic phosphorus atom".[2] The mutagenicity of compounds having structures II and III has received no attention, so far as we are aware, despite the use of such compounds as pesticides, lubricants, antioxidants,

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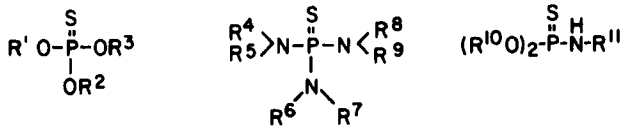


I II III

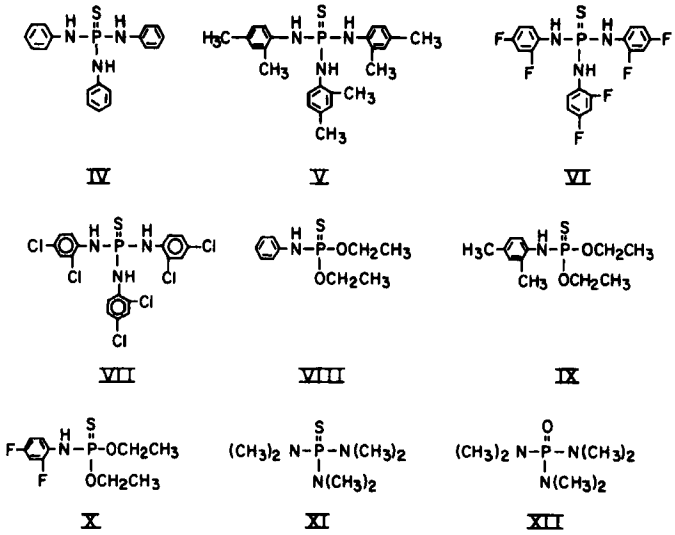
FIGURE 1 Classes of thionophosphorus compounds studied.

and agents for fireproofing. This paper reports a study of the mutagenicity of representative compounds with the structures II and III.

RESULTS

Of the group IV–XII, compounds V–VII, IX and X are reported here for the first time. Compound XI was prepared by modifying reported methods, IV and VIII were synthesized by our general methods described, and XII was commercial material.

Compounds IV-XII were assayed over a five-log concentration range from 1 μg to 10 mg per plate for mutagenicity by their capability for inducing back mutation of the histidine operon in the base-pair mutation (Strain TA 100) and frame-shift mutation (Strain TA 98) of *Salmonella typhimurium*. With each strain, assays were done both with and without metabolic activation by a S-9 liver homogenate with exogenous NADP (rat-liver homogenate induced by Arochlor 1254). The tris(aryl)thiophosphoramides IV–VII were not mutagenic under any of the four conditions. Of the three phosphoramidothioates VIII–X, IX was not mutagenic. The phenyl compound VIII was inconsistently mutagenic with metabolic activa-



IV V VI

VII VIII IX

X XI XII

FIGURE 2 Compounds tested for mutagenicity.

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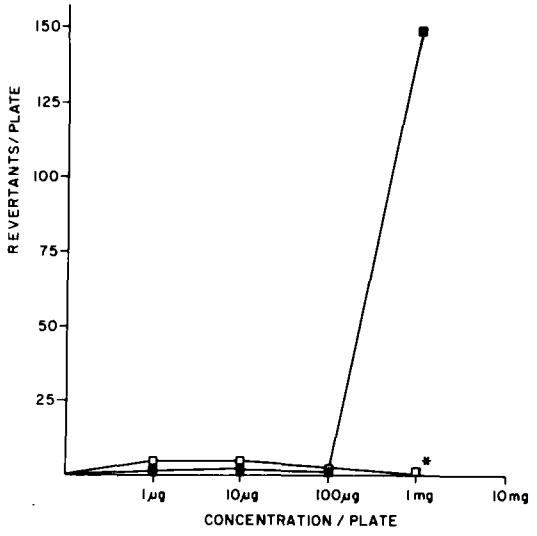

FIGURE 3 Mutagenesis induced in *Salmonella typhimurium* by O,O-diethyl N-(2,4-difluorophenyl)phosphoramidothioate (X). □, Strain TA 100 without S-9 incubation; ■, Strain TA 100 with S-9 incubation. Data are reported as revertants per plate corrected for spontaneous reversion rate. Each point represents the mean of three observations from one experiment. The asterisk adjacent to the experimental data point for the highest concentration of X without S-9 incubation indicates an acute toxicity of X at this concentration (i.e., the bacterial lawn was absent).

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tion but only against Strain TA 98 and only at levels that bordered on toxicity (100 μg–1 mg/plate). The difluorophenyl compound X on the other hand did prove to be mutagenic. Compound X was consistently mutagenic following S-9 activation, although only for the base-pair mutation (Strain TA 100) and only at high concentrations of X (on a single occasion mutagenicity was observed in both Strain TA 100 and TA 98 following metabolic activation). The results from a typical experiment with X are shown in Figure 3; these results are typical in that mutagenicity is observed only at the highest concentration of X that was not acutely toxic to the test organism. The results shown are conservative; Compound X did induce mutations on occasion which were several times greater than illustrated in Figure 3. At concentrations of X that induced mutation following S-9 activation (presumably involving conversion of P═S to P═O), there was consistently observed acute toxicity to the tester *Salmonella* strains by non-metabolized X (since the bacterial lawn, although present, was less than usual). The hydrolysis product of X, 2,4-$F_2C_6H_3NH_2$, was not mutagenic; indeed, it decreased the number of spontaneous revertants.

With the aliphatic thioamide XI and its oxygen counterpart (XII), equivocal results were obtained. In two of five separate evaluations, both XI and XII led to colony counts that were approximately twice background, but neither was significantly mutagenic in three other trials, suggestive of a very delicate mechanism for mutagenesis. In contrast to X, the P═O compound (XII) at 500 μg/plate was more acutely toxic to *S. typhimurium* than was the P═S compound (XI).

DISCUSSION

The group IV–X includes representatives of thiophosphoramides containing three bulky aryl groups (IV–VII), together with phosphoramidothioates containing only one bulky aryl group and two relatively small alkyl groups (VIII–X). Each of these two classes contains representatives with hydrogen as a reference aryl substituent (IV, VIII), with electron-donating substituents (V, IX), and with electron-withdrawing groups (VI, VII, X). The mutagenic proclivities of these seven aryl derivatives should provide clues as to the effects on mutagenesis one may expect of both bulk and electron supply. The last compound (XI) permits comparison of the effect of alkyl groups with aryl groups in thiophosphoramides, with XII being included for an estimate as well of the relative effects of P═S and P═O linkages.

In a general way, the results with the aryl compounds IV-X fall into the pattern of substituent effects found for esters of Type I,[2] where for mutagenesis two groups were relatively small. With the esters, the third group was a good "leaving group", a quality enhanced by electron withdrawal owing to enhanced stability of the departing anion. Hence the inactivity of IV-VII, having three bulky aryl groups, is consistent with inactivity found for Type-I esters where $R^1 = R^2 = R^3 =$ Ph, or 2,4,6-$(CH_3)_3C_6H_2$, or even 3,5-$Cl_2C_6H_3$ (despite electron withdrawal by Cl in the latter).

With the phosphoramidothioates VIII-X, electron withdrawal seems important, as it was with the previous esters.[2] Thus the inactivity of IX contrasts with the activity of X and the marginal and inconsistent activity of VIII (S-9 activation of VIII and X presumably converts the P═S linkage into a P═O linkage of the actual mutagen; cf. ref. 3). Thus some parallel in mutagenesis was observed with the following mutagenic esters of Type I.[2] Also consistent was the

(a) $R^1 = 4\text{-}NO_2C_6H_4$ (electron-withdrawing); $R^2 = R^3 = CH_3$ or CH_2CH_3 (small groups)

(b) $R^1 = R^2 = R^3 = F_3CCH_2$ (electron-withdrawing and small)

marginal mutagenesis found with the reference phenyl ester where in Structure I $R^1 =$ Ph and $R^2 = R^3 = CH_2CH_3$. It is consistent with these results for esters, therefore, that X is mutagenic (despite only relatively weak electron withdrawal from the ortho and para fluorine atoms), that VIII is only marginally mutagenic, and that IX is not mutagenic at all.

With alkyl esters of Type I, mutagenicity was not observed when the alkyl groups were large and not electron-withdrawing [$R^1 = R^2 = R^3 = C(CH_3)_3$]. Since it will be recalled that XI was mutagenic in only two of five trials, the apparently marginal mutagenicity of the N-alkyl thiophosphoramide XI seems consistent with results with the esters [$(CH_3)_2N$ is both bulky and a poor leaving group].

It also was of interest to compare the alkyl thiophosphoramide XI with its oxygen counterpart XII, since XII has carcinogenic proclivities.[4] The behavior of XII resembled that of XI in leading only to marginal mutagenicity in our assay, although XII has led to mutations or neoplasia in other assay systems.[5] On the other hand, another study showed that only one of fifteen other investigators

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found XII to be mutagenic in the *Salmonella* assay.[6] Ashby has suggested that since XII is active in several mutagenesis-related assays *other* than with Salmonella, aberrant results with *Salmonella* may be attributed to insufficient metabolic activation. It should be recognized, however, that since the mutagenicity of XI and XII may result from formation of formaldehyde,[7] mutagenesis by XI and XII may be unrelated to the present work. We conclude that XII probably is mutagenic but that it requires stringent metabolic activation conditions and that the thiono counterpart XI probably owes its effect to biotransformation to XII.

Although guidelines to mutagenicity at present must be based on too few compounds to provide more than clues for further correlations, the following conclusions seems reasonable: (1) The amides appear to be no more mutagenic than the esters and probably are less so (cf. the marginal mutagenicities of both VIII and I with $R^1 = C_6H_5$ and $R^2 = R^3 = CH_2CH_3$, as well as the marginal mutagenicity of the alkyl thiophosphoramide XI). Thus, while six of eleven phosphorothioate esters seemed *unquestionably* mutagenic at ca. 500 μg per plate,[2] only three of eight of the present thioamides were *marginally* mutagenic and then only at high concentrations that bordered on cell toxicity. The conclusion as to lower mutagenic hazard with the amides is not surprising since one would expect the amines to be poorer leaving groups than phenols or alcohols and therefore less susceptible to attack by nucleophilic biomacromolecules. (2) As with esters of Type I, amides of Type III seem most likely to be mutagenic when both of the groups R^{10} of Structure III in Figure 1 are small enough to permit facile attack of a biomacromolecule on the electrophilic phosphorus atom. The capability of the third group (NR^{11} of Structure III in Figure 1) for strong electron withdrawal also seems important, although the third group of the amides need not be a good leaving group, in contrast to the third group of the esters (perhaps because of a different mechanism of action).

The greater mutagenicity of metabolically activated VIII and X most likely finds its explanation in the conversion of P═S to P═O,[3] owing to greater electronegativity of oxygen vs. sulfur and the resultant greater susceptibility to nucleophilic attack on the electrophilic phosphorus atom by biomacromolecules. A similar explanation was advanced and discussed for the mutagenicity of TEPA without metabolic activation, in contrast to the need for S-9 enzymatic activation to engender mutagenicity of thio-TEPA.[8] Since the concentration that leads to acute metabolic toxicity of the thiophosphoramides is similar to that necessary for S-9 mediated conversion to a mutagenic species, it seems unlikely that the representative compounds tested pose a serious "silent" hazard to population groups exposed to the compounds (i.e. mutagenicity seems likely to occur only near concentrations that would in any event be toxic).

EXPERIMENTAL

Materials

Figure 2 shows the compounds IV-XII that were tested for mutagenicity. Of these, hexamethylphosphoramide (XII) was a commercial sample (which was used for comparison with its thiono counterpart, XI). The other compounds were synthesized as described below. Melting points were determined by using a Thomas-Hoover stirred-liquid apparatus and are corrected. ^{1}H NMR spectra were recorded in $CDCl_3$ or acetone-d_6 on a JEOL JNM-MH-100 or FX-90Q spectrometer using either

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Me_4Si or $CDCl_3$ as internal standards, unless otherwise mentioned. ^{13}C NMR spectra were recorded on a JEOL FX-90Q spectrometer (with $CDCl_3$ or acetone-d_6 as solvent and standard), and ^{31}P NMR spectra were obtained with a Varian XL-100 spectrometer with acetone-d_6 as solvent and 85% H_3PO_4 as an external reference. All NMR spectra are reported in parts per million (δ). The HRMS result was kindly provided by Dr. B. J. Sweetman, Dept. of Pharmacology, by use of a VG 70/250 HF GC/MS mass spectrometer; funds were provided by the N.I.H. Division of Research Resources Grant No. RR 01688. Starting materials, unless otherwise noted, were obtained from the Aldrich Chemical Co. or the Alfa Division of the Ventron Corporation and were used without further purification. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Solvents were removed under reduced pressure by using a rotary-flask evaporator. Thin-layer chromatography (TLC) was done by using Eastman Chromagram sheets of silica gel, cat. no. 13181, with 15–30% EtOAc in hexane. Flash chromatography was carried out using the procedure of Still, Kahn and Mitra with Baker "Flash Chromatography Grade" silica gel (200 g in a 50-mm column) and with EtOAc-hexane mixtures as eluting solvents.[9] Reactions were routinely carried out under Ar using oven-dried glassware. The term "mol prop" represents molar proportions.

General Method A (for IV-VI). One mol prop of $PSCl_3$ (thiophosphoryl chloride) was added dropwise to a solution of ca. six mol prop of the appropriate aniline dissolved in ca. 2 ml of toluene per gram of the aniline with stirring. The mixture then was heated under reflux for 2-4 days with stirring (until TLC or NMR showed absence of the amine starting material). After being cooled, the mixture was washed once with ca. an equal volume of H_2O, twice with an equal volume of ice-cold 1 N HCl, and again with water. The organic layer was dried ($MgSO_4$), filtered by gravity, and concentrated. Particulars are given below for IV-VI.

In the synthesis of *N,N',N''*-trisphenylthiophosphoramide (IV), 40 mmol of $PSCl_3$ and 250 mmol of aniline after 2 days of reflux gave 12.6 g of off-white crystals, which after two recrystallizations from CCl_4 gave 9.2 g (68%) of colorless IV: mp 152–153°C (lit.[10] mp 153–154°C); ^1H NMR ($CDCl_3$) δ 7.24–7.08 (m, 15H), 4.50 (br d, 3H); ^{13}C NMR ($CDCl_3$) δ 139.22, 129.28, 122.83, 119.70 (d).

With *N,N',N''-tris*(2,4-*dimethylphenyl*)*thiophosphoramide* (*V*), 50 mmol of $PSCl_3$ and 305 mmol of 2,4-dimethylaniline after 4 days of reflux gave 14.6 g (69%) of brown crystals. This product (V) was recrystallized once from hexane and once from CCl_4 to give a light brown solid: mp 148.5–149.5°C; ^1H NMR ($CDCl_3$) δ 7.40 (d, 3H), 6.92 (d, 6H), 4.94 (d, 3H), 2.28 (s, 9H), 2.19 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.07 (d), 132.72 (d), 131.45, 128.26 (d), 127.27 (d), 120.49 (d), 20.58, 17.68; ^{31}P NMR (acetone-d_6), 47.55. Anal. Calcd. for $C_{24}H_{30}N_3PS$: C, 68.06; H, 7.14; S, 7.57. Found: C, 68.25; H, 7.14; S, 7.78.

In the preparation of *N,N',N''-tris*(2,4-*difluorophenyl*)*thiophosphoramide* (*VI*), 25 mmol of $PSCl_3$ and 155 mmol of 2,4-difluoroaniline after 3 days of reflux gave 6.74 g (60%) of VI as a dark purple glass: ^1H NMR ($CDCl_3$) δ 7.75–7.36 (br m, 3H), 7.04–6.84 (br m, 6H), 6.64 (d, 3H); ^{13}C NMR ($CDCl_3$) δ 158.56 (ddd), 154.08 (ddd), 122.86 (m), 111.22 (dd), 104.06 (dd). Anal. Calcd. for $C_{18}H_{12}F_6N_3PS$: C, 48.33; H, 2.71; S, 7.17. Found: C, 48.09; H, 3.03; S, 7.13.

General Method A gave an insignificant yield of *N,N',N''-tris*(2,4-*dichlorophenyl*)*thiophosphoramide* (*VII*). Compound VII therefore was obtained by addition to 62 mmol of NaH in 10 ml of dry dioxane of 63 mmol of purified 2,4-dichloroaniline (recrystallized twice from Me_2CO to a constant melting point of 63–64°C) in 40 ml of dry dioxane. The reaction vessel then was placed in an ultrasonic cleaner (Branson Model B-220, 117 volts, 125 watts), partially filled with H_2O, and a reflux condenser was attached. The sonication device then was activated for about 5 min (no apparent reaction). The instrument was turned off, and 20 mmol of $PSCl_3$ in 10 ml of dry dioxane was added to the reaction mixture without sonication. After the addition was completed, the ultrasonic cleaner was reactivated. The course of the reaction was followed by ^1H NMR and TLC. After two days of sonication (during which the temperature rose to 50°C) an additional 62 mmol of NaH was added to the mixture. After 3.5 days more of sonication, the ultrasonic cleaner was deactivated. The product mixture then was diluted with two volumes of $CHCl_3$, and excess NaH was destroyed by cautiously adding MeOH until no gas evolution was observed. The $CHCl_3$ solution was washed once with H_2O, twice with ice-cold 1 N HCl, and again with H_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give 14.20 g of dark reddish-brown solid. Nine grams of this solid then was purified further by flash chromatography on a 50-mm i.d. column (230 g of silica gel, 20:80 EtOAc-hexane). Two major fractions were collected; the first showed 2 spots on TLC at $R_f = 0.70$ (major) and 0.60 (minor), and the second showed 2 spots at 0.70 (minor) and 0.60 (major). The compounds were isolated in yields of 4.5 g (65% yield of VII) and 0.70 g respectively. A portion (300 mg) of the first fraction then was chromatographed on a preparative TLC plate (10% EtOAc-hexane) to give 225 mg (75% recovery, 49% overall yield) of VII as a brown solid: m.p. 119–120°C; ^1H NMR ($CDCl_3$) δ 7.60–7.00 (m, 9H), 5.80 (d, 3H); ^{13}C NMR ($CDCl_3$) δ 134.37, 128.95, 128.33, 127.98, 124.90 (d, $J = 9.16$ Hz), 121.14 (d, $J = 3.05$ Hz); ^{31}P NMR (acetone-d_6) δ 46.2. Anal. Calcd. for $C_{18}H_{12}Cl_6N_3PS$: mol. wt., 542.86253. Found (HRMS): mol. wt. 542.86265.

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General Method B (For VIII-X). One mol prop of $(EtO)_2PSCl$ (diethyl chlorothiophosphate) in ca 2–4 ml of toluene or benzene per gram of the aniline was added dropwise with stirring to a solution of one mol prop of the appropriate aniline and one mol prop of Et_3N in ca 2 ml of toluene per gram of the aniline. The solution was heated under reflux for 1–3 days with stirring and then was cooled [reflux was continued until TLC showed no spot for $(EtO)_2PSCl$]. The mixture then was washed once with an equal volume of H_2O, twice with a equal volume of ice-cold 1 N HCl, and again with H_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated. Particulars for VIII-X were as follows:

In the synthesis of *O,O-diethyl N-phenylphosphoramidothioate* (*VIII*), 100 mmol each of $(EtO)_2PSCl$, Et_3N, and aniline after 72 hr of reflux in toluene gave 16.9 g (69%) of VIII as a viscous orange-red oil. A portion (8.1 g) was purified by flash chromatography with 20:80 EtOAc-hexane for development. Combination of the remainder of the 16.9 g after similar purification gave a total of 9.40 g (38%) of VIII as yellow oil: $n_D^{24.5}$ 1.5445 (lit. n_D^{20} 1.5502;[11] ^1H NMR ($CDCl_3$) δ 7.40–7.00 (m, 5H), 5.87 (d, 1H), 4.35–4.00 (m, 4H), 1.31 (t, 6H); ^{13}C NMR ($CDCl_3$) δ 139.33 (d), 129.03, 121.88, 117.62 (d), 63.03 (d), 15.59 (d).

In the preparation of *O,O-diethyl N-(2,4-dimethylphenyl)phosphoramidothioate* (*IX*), 100 mmol each of $(EtO)_2$ PSCl, Et_3N, and 2,4-dimethylaniline after 64 h of reflux in toluene gave 20.4 g (75%) of orange-red oil. After purification by flash chromatography as described for VIII (but with 5:95 EtOAc-hexane), TLC analysis showed more than one spot. The oil therefore was washed again with ice-cold HCl (30 ml of 0.1 N) and water and subjected again to flash chromatography (with 3:97 EtOAc-hexane) to give 4.50 g (16%) of IX as an oil. Preparative TLC of 1 g of this oil on a 1000-μm Brinkmann PK6F silica-gel plate with 5:95 EtOAc-hexane then led to a pale yellow semisolid, which after recrystallization from hexane with dry-ice cooling gave 0.15 g of IX with mp 31–32°C: ^1H NMR ($CDCl_3$) δ 7.18 (d, 1H), 6.95 (d, 2H), 5.05 (d, 1H), 4.10 (m, 4H), 2.24 (s, 3H), 2.19 (s, 3H), 1.28 (t, 6H); ^{13}C NMR ($CDCl_3$) δ 135.00 (d), 131.63 (d), 131.01, 127.17, 125.58 (d), 118.43 (d), 62.93 (d), 20.31, 17.41, 15.53 (d). Anal. Calcd. for $C_{12}H_{20}NO_2PS$: C, 52.73; H, 7.38; S, 11.73. Found: C, 52.67; H, 7.26; S, 12.00.

In the synthesis of *O,O-diethyl N-(2,4-difluorophenyl)phosphoramidothioate* (*X*), 50 mmol each of $(EtO_2)PSCl$, Et_3N, and 2,4-difluoroaniline after ca. 18 hr of reflux in benzene gave X as a dark purple oil. Short-path distillation (6-cm column) then gave 7.85 g (56%) of X as a colorless oil: bp 108–110°C (0.1 torr); n_D^{23} 1.5070; ^1H NMR ($CDCl_3$) δ 7.63–7.36 (m, 1H), 7.05–6.87 (m, 2H), 5.66 (d, 1H), 4.40–4.07 (m, 4H), 1.33 (t, 6H); ^{13}C NMR ($CDCl_3$) δ 155.26 (d of d), 151.81 (m), 123.70 (m), 119.8 (m), 110.83 (d of d), 103.50 (d of d), 63.30 (d), 15.49 (d); ^{31}P NMR (acetone-d_6) δ 46.8 (br, m). Anal. Calcd. for $C_{10}H_{14}F_2NO_2PS$: C, 42.70; H, 5.02; S, 11.40. Found: C, 42.39; H, 5.27; S, 11.65.

Hexamethylthiophosphoramide (*XI*). Tris(dimethylamino)phosphine was prepared, essentially as reported,[12] by slowly bubbling Me_2NH (250 mmol) into a solution of 5.36 g (39 mmol) of phosphorus trichloride in 75 ml of anhydrous ether at 8–10°C with stirring and then stirring at ca. 25°C overnight. The solution then was filtered using a sintered-glass funnel and concentrated to 50 ml. Elemental sulfur (1.28 g, 40 mmol) was added during ca. 5 min (to prevent a vigorous exotherm) with stirring. After 0.5 hr, an additional 50 mmol of sulfur was added. The reaction mixture was stirred at ca. 25°C overnight and then concentrated to give a viscous red oil. When attempts to purify this oil by short-path distillation under reduced pressure were unsuccessful, the oil was flash chromatographed with 20:80 EtOAc-hexane. There was obtained 3.65 g (48%) of XI as a reddish solid, along with small amounts of elemental surfur and a third component (presumably the unreacted phosphine or the corresponding phosphate): mp 24–26°C (lit.[13] mp 29°C); ^1H NMR ($CDCl_3$) δ 2.66 (d) [lit.[14] ^1H NMR δ 2.60 (d)]; ^{13}C NMR ($CDCl_3$) 35.84(d).

Assays for Mutagenicity

Ames assays for mutagenicity were performed largely as published,[15] together with minor modifications previously described.[8] DMSO was used as a solvent for all compounds; these were titered over a five-log concentration range (1, 10 and 100 μg and 1, 10 mg). Incubations with *Salmonella typhimurium*, strain TA 98 and TA 100 were made at the stated concentrations at 37°C for 1 hr in a mixture containing in final concentration 0.07 M Na phosphate (pH 7.4), 5.6 mM $MgCl_2$, 2.8 mM NADP, 3.5 mM D-glucose-6-phosphate, and 14% (w/v) Aroclor 1254-induced male Sprague-Dawley liver homogenate, or sterile water in the non-S9 incubations; plating in agar, incubation and scoring were done as reported.[8] 2-Aminoanthracene was used routinely as a positive control (i.e., >2000 revertants per plate for each strain with S9 activation at a concentration of 2 μg per plate). Acute toxicity was determined by direct observation of the bacterial lawn. All results were corrected for spontaneous revertants in control incubates (i.e., 100–120 for TA 100 and 30–50 for TA 98). As before,[2] compounds were considered mutagenic only when the colony count was at least twice that with solvent controls.

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REFERENCES AND NOTES

1. (a) Paper 7: J. H. Hillhouse, I. A. Blair and L. Field, *Phosphorus Sulfur,* **26,** 169 (1986); (b) presented in part at the 34th Southeastern Regional Meeting of the American Chemical Society (Birmingham, Ala., Nov. 19, 1982) and the 22nd Annual Meeting of the Society of Toxicology (Las Vegas, Nev., March, 1983); (c) further details may be found in the doctoral dissertations of A.P.B. (1983) and J.S. (1985).
2. A. P. Breau, W. M. Mitchell, J. Swinson and L. Field, *J. Toxicol. Environ. Health,* **16,** 403 (1985).
3. R. A. Neal, in E. Hodgson, J. R. Bend and R. M. Philpot (Eds.), *Reviews in Biochemical Toxicology* (Elsevier/North Holland, New York, 1980), Vol. 2. pp. 131–171.
4. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man:* "Hexamethylphosphoramide" (International Agency for Research on Cancer, Lyon, 1977), Vol. 15, pp. 211–222.
5. (a) J. Palmquist and L. E. Lachance, *Science,* **154,** 915 (1966); (b) R. J. Sram, *Folia Biol. (Praha),* **18,** 139 (1972); (c) J. A. Zapp, Jr., *Am. Ind. Hyg. Ass. J.,* **36,** 916 (1975); (d) K. P. Lee, H. J. Trochimowicz and J. W. Sarver, *Lab. Invest.,* **36,** 344 (1978); (e) G. Mueller and K. Narpoth, *Arbeits med. Kollog. Ber. Jahrestag,* **18,** 83 (1978).
6. J. Ashby, *Progr. Mutat. Res.* **1,** 112 (1981).
7. A. R. Jones and H. Jackson, *Biochem. Pharmacol.,* **17,** 2247 (1968).
8. A. P. Breau, L. Field and W. M. Mitchell, *Cell Biol. Toxicol.,* **1,** 13 (1984).
9. W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.,* **43,** 2923 (1978).
10. A. C. Buck, J. D. Bartleson and H. P. Lankelma, *J. Am. Chem. Soc.,* **70,** 744 (1948).
11. M. I. Kabachnik, V. A. Gilyarov and R. V. Kudryavtsev, *Zh. Obshch. Khim.,* **35,** 1476 (1965); *Chem. Abstr.,* **63,** 14733 (1965).
12. L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis* (John Wiley and Sons, New York, 1967), Vol. 1, p. 43.
13. H.-J. Vetter and H. Nöth, *Chem. Ber.,* **96,** 1308 (1963).
14. A. H. Cowley and R. P. Pinnell, *J. Am. Chem. Soc.* **87,** 4454 (1965).
15. (a) J. McCann, E. Choi, E. Yamasaki and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.,* **72,** 5135 (1975); (b) B. N. Ames, J. McCann and E. Yamasaki, *Mutat. Res.,* **31,** 347 (1975).

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