IMPROVED TECHNIQUES FOR SEMI-MICRO SYNTHESIS OF RADIOISOTOPE LABELLED THIOPHOSPHATE PESTICIDES

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SUMMARY

Radiolabelled ethanol can be incorporated into the organophosphate molecule by heating $PSCl_3$ with the labelled alcohol. Methanol can also be incorporated in this manner, but with lower yields. The O, O-dialkyl phosphorochloridothionates were produced by treating O-alkyl phosphorodichloridothionate with the appropriate sodium alkoxide. Coupling the O, O-dialkyl phosphorochloridothionate to a substituted phenol to form the pesticidal compounds was facilitated by the catalytic effect of silver ions. The catalyzed coupling reaction was found to be compatible with hydroxylated heterocyclic compounds, and with phenols containing nitro-, mercapto-, alkyl-, and cyano-substituents. The reaction was not successful, however, with chloro-substituted phenols. The coupling reaction was also found to be useful in synthesizing EPN, a phosphonothionate pesticide. The coupling reaction does not appear to be useful for the synthesis of oxons, the analogous phosphate compounds.

INTRODUCTION

Methods described for semi-micro organophosphate synthesis are frequently complicated by side reactions and/or low yields. During the design of an improved procedure for the synthesis of radiolabelled diazinon we have developed a process of more general applicability for the incorporation of radiolabelled alcohols into the thiophosphoryl center, and the coupling of the hydroxy-substituted pyrimidine or phenol to the phosphorochloridothioate diester.

A starting material frequently used in the synthesis of thiophosphate pesticides is phosphorus pentasulphide [1a,2]. This reagent is converted to the

O,O-dialkyl phosphorochloridothioate by refluxing in an alcohol with subsequent chlorination using chlorine or sulphuryl chloride [1a,2]. Although this method is reasonably straightforward and frequently utilized in syntheses of labelled pesticides, it involves excessive handling and overall yields are about 30%. An alternate, one step approach utilizing thiophosphoryl chloride as starting material has been reported [1b], with a yield of about 50%[3]. We have modified this procedure to obtain somewhat better yields with the additional advantage that there is no dilution of the labelled alcohol during incorporation into the thiophosphoryl center. This reaction is shown in Figure 1.

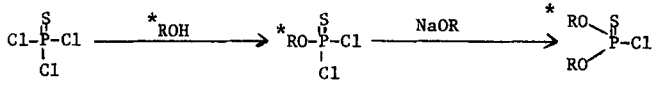

Figure 1. Two step procedure for the synthesis of radiolabelled O,O-diethyl phosphorochloridothionate using thiophosphoryl chloride as starting material.

In our hands, the coupling of the hydroxy-pyrimidine to the thiophosphoryl center is the reaction which decreases the overall yield during diazinon synthesis. The problems encountered here are difficult to alleviate due to the limited number of ways this reaction can be accomplished. The coupling of aromatic phenols to the thiophosphoryl center is generally carried out by stirring the phenol and the O,O-dialkyl phosphorochloridothionate at elevated temperature in solvents such as benzene, toluene, or methyl ethyl ketone in the presence of a proton acceptor such as an amine or metal carbonate[4a,5]. We have preferred to avoid long reaction times at elevated temperature, since these conditions are known to facilitate rearrangement of phosphorothionate triesters to the correponding S-alkyl and S-phenyl isomers[4b]. The most obvious approach to improve the yield from this reaction is to increase the nucleophilic character of the phenol. This may be done by converting the phenol to the corresponding phenoxide anion. The use of strong nucleophiles, however, opens the possibility of O-dealkylation of both the starting material and the product resulting from nucleophilic attack on the number 1 carbon of the alkoxide sub-

stituent of the O,O-dialkyl phosphorothionate[6,7]. A less obvious approach to the problem is to increase the reactivity of the phosphorochloridothionate. Nucleophilic substitution at phosphorochloridothionate diester and related compounds takes place through an S_N2 bimolecular mechanism[8]. However, under standard conditions this effect did not produce a significant difference. A second approach to increasing the reactivity of the phosphorochloridothionate center is through metal catalysis, the method found to be most fruitful in this study. Silver ions are known to coordinate strongly with both sulphur and halogens[9], and have been found to catalyze solvolysis of a number of halogen and sulfur containing compounds[10,11,12]. Silver ions have been found to accelerate the hydrolysis of several organothiophosphate triesters[13,14] through an interaction with the sulphur atom.

Although all the synthesis in this study did not utilize radioisotopes, conditions, procedures, and apparatus were used to simulate labelled syntheses.

EXPERIMENTAL

Reagents. 1-^{14}C-ethanol was obtained from INC, Chemical and Radioisotope Division, and the 6-hydroxy-4-isopropyl-2-methyl pyrimidine was obtained from CIBA-Geigy Chemical Corporation. Thiophosphoryl chloride was obtained from Ventron Corporation, Alfa Products. All other reagents were obtained from Aldrich Chemical Company.

Sodium alkoxides were prepared fresh by addition of sodium metal to the appropriate absolute alcohol.

O-ethyl phenylphosphonochloriodothionate was prepared from phenylphosphonodichloridothionate by the method of Hoffmann[15].

Synthesis of 1-(^{14}C-ethoxy)-O,O-diethyl phosphorochloridothionate. The vial in which the ^{14}C-ethanol was received (29 µl; specific activity 2.0 µCi/µM) was utilized as the reaction vessel as shown in Figure 2. The bottom of the vial was placed in a dry ice-ethanol bath for 20 minutes to condense all vaporized material. The seal of the vial was broken and 170 µl (1.65×10^{-3} moles) of freshly distilled thiophosphoryl chloride was placed in the bottom of the vial by use of a hypodermic syringe equipped with a long needle. The vial

was fitted with an ice-filled styrofoam cup and protected from moisture as shown in Figure 2, placed in a hot water bath, and heated to 60°C. After

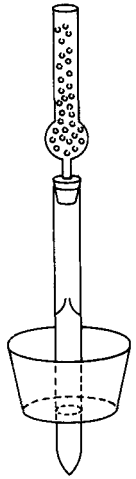

Figure 2

4 hours of 60°C, 67 µl of unlabelled ethanol were added to the vial (for a total of 1.65×10^{-3} moles) and the resulting mixture was heated at 60°C for 3 hours. The contents of the vial were taken up in 2 ml of petroleum ether and transferred to a 10.0 ml two necked flask containing a small magnetic stirring bar and equipped with a drying tube. The flask was placed in an ice bath and vigorously stirred while a slight excess (1.8×10^{-3} moles) of 2 N $NaOC_2H_5$ in ethanol was added in a dropwise manner. After the addition was completed, the reaction mixture was stirred for an additional 5 minutes and then transferred to a small test tube. The reaction flask was rinsed twice with small amounts of petroleum ether and the rinsings combined in the test tube. The petroleum ether solution was extracted with 6 ml of cold 0.1N aqueous $NaHCO_3$. The organic phase was removed and the aqueous phase extracted with a small amount of petroleum ether. The organic phases were combined, dried over $CaCl_2$, and the solvent removed under dry nitrogen to give 245 mg of material which was found to be homogeneous on TLC (silica gel; solvent system 4:1 heptane: acetone) and the O,O-diethyl phosphorothioic acid derived from the O,O-diethyl phosphoro-

chloridothionate was characterized by it's IR spectrum[16]. The yield, based on $PSCl_3$, was 78%.

Synthesis of O,O-dimethyl phosphorochloridothionate. This synthesis was carried out in a similar manner to the synthesis of the analogous diethyl compound described above. The first step of the reaction, the incorporation of the alcohol into the $PSCl_3$ molecule, was carried out in a tapered 10 ml centrifuge tube to simulate the vial in which the radiolabelled alcohols are received. This step was carried out at a lower temperature (50°C) due to the higher volatility of methanol. The next step of the reaction, the introduction of the second methoxy substituent to form O,O-dimethyl phosphorodichloridothionate, was similar to the synthesis of the analogous diethyl compounds, except that sodium methoxide was used. The product was homogenous on TLC (silica gel; solvent system 4:1 heptane: acetone) and its structure was verified by IR spectroscopy [16]. The yield was 65% based on $PSCl_3$.

Synthesis of pesticides by coupling substituted phenols to O,O-dialkyl phosphorochloridothionate or O-ethyl phenylphosphonochloridothionate. In a typical reaction, a slight excess of the appropriate phenol was added to a small round bottomed flask containing 1.65 mmoles of the phosphorochloridothionate. Four ml of a two phase solvent system (benzene/water, 1:1, V/V). 600 mg of Ag_2CO_3 were added and the resulting mixture stirred at room temperature for a period of time as shown in Table 1. During the synthesis of EPN, use of ethyl acetate as the solvent was found to give better yields than the 2 phase benzene/water system. The progress of the reactions was monitored by chromatographing 5 µl aliquots of the reaction mixture on silica gel TLC (solvent system 4:1 heptane: acetone). The reactions were stopped after all of the phosphorochloridothionate had been consumed. The contents of the reaction flask were transferred to a small test tube and partitioned twice against 4 ml amounts of 0.1N aqueous $NaHCO_3$. The test tube was centrifuged to facilitate separation of the phases. The organic phase was removed and the benzene evaporated under a stream of nitrogen at 40°C to give the crude product. It was sometimes necessary to extract the residual material several times with benzene to recover product absorbed to the precipitated silver salts and unreacted

Ag_2CO_3. Sumithion and diazinon were particularly difficult to recover. The crude products were somewhat discolored, but the IR spectra obtained were identifical to reported spectra. TLC analysis indicated that the crude products were generally contamined by small amounts of unreacted phenols. A high degree of purity can be attained by a simple cleanup on preparative TLC.

All chromatographic systems utilized silca gel GF-254 (Brinkmann) as the stationary phase.

The structures of all pesticides synthesized were verified by co-chromatography with authentic standards on TLC and by their IR spectra with reference to published spectra as shown in Table 1, except O,O-diethyl O-(4-methylthio)-phenyl phosphorothionate, for which no IR spectrum could be located. This compound was tentatively identified by interpretation of its IR and NMR (proton) spectra. The IR spectrum was typical of an aryl dialkyl phosphorothionate triester. The NMR spectrum was more diagnostic, giving the following information: quartet at τ 2.9 (4H) corresponding to the protons of the aromatic ring[17a], a split quartet at τ 5.9 (4H) corresponding to the methylene protons of the ethoxy groups[18], a singlet at τ 7.7 (3H) corresponding to the protons of the methylthio groups [17b], and a triplet at τ 8.75 (6H) corresponding to the methyl protons of the ethoxy substituents[18].

DISCUSSION

The direct incorporation of labelled alcohols into thiophosphoryl chloride and subsequent treatment with sodium alkoxide to form O,O-dialkyl phosphorochloridothionate was not extensively investigated, but results with ethanol and sodium ethoxide, and unlabelled methanol and sodium methoxide were good. The synthesis of radiolabelled diazinon gave a final specific activity of 0.55 mCi/mmole which represents an incorporation of 82% of the labelled ethanol. The incorporation of methanol into thiophosphoryl chloride was less productive than when ethanol was used, resulting in a lower but still useful incorporation. O,O-diethyl and dimethyl phosphorochloridothionates are frequently utilized in pesticide synthesis and seemed most pertinent to this study. Other alcohols and alkoxides might also give good results.

The incorporation of the alcohol is augmented by an excess of the thiophosphoryl chloride. Therefore, the ^{14}C-ethanol and thiophosphoryl chloride were reacted together for several hours before addition of the stoichiometric balance of ethanol (unlabelled) to maximize the incorporation of the label. Although small amounts of unreacted thiophosphoryl chloride and O,O-dialkyl compounds could be detected in the reaction mixture by IR spectrometry, neither are serious contaminants, i.e., the dialkyl phosphorochloridothionate is the desired penultimate intermediate in the synthesis of pesticides, and the unreacted thiophosphoryl chloride is converted directly to the desired compound by the use of an excess of the sodium alkoxide. An excess of the alkoxide is also necessary to neutralize HCl produced during the reaction. Formation of undesired trialkylated phosphorothionates by excess alkoxide is avoided by cooling the reaction mixture. Further simplification of this step might be accomplished by pipetting the alkoxide solution directly into the ampoule in which the initial step of the synthesis was executed.

Reactions utilizing less than approximately 1 mmole of thiophosphoryl chloride starting material do not seem practical due to difficulties in handling microlitre and milligram quantities of reagents and products. In addition, the minuteness of the reaction is limited by the need of an excess of thiophosphoryl chloride in the first step to facilitate uptake of the label.

Coupling of substituted phenols to phosphorochloridothionates in the presence of silver carbonate appears to be a widely useful reaction. High yields were obtained with little interference by side reactions. The reaction works well with both O,O-dimethyl and O,O-diethyl phosphorochloridothionates. The success realized in the synthesis of EPN (O-ethyl O-p-nitrophenyl phenylphosphonothioate) indicates the phosphonothionates can also be prepared by this method. Several phenols containing various substituents cause no interference. Nitro-, thioalkyl-, and cyano-substituted phenols couple well. Hydroxylated nitrogen heterocycles couple to produce compounds such as diazinon. However, the coupling reaction is not successful with 2,4-dichloro- or 2,4,5-trichlorophenols, possibly due to interaction of silver ions with the chlorine substituents. This reaction does not seem to be of any value in the synthesis

of oxons (analogous P=O compounds). Attempts were made to prepare the oxygen analogs of parathion and diazinon by coupling the phenol or the hydroxypyrimidine to O,O-diethyl phosphorochloridate using Ag_2CO_3. Aqueous and non-aqueous solvent systems were investigated, but in no case was any desired product isolated.

The solvent system utilized in the coupling of the phenol to the phosphorochloridothionate was found to be important to the yield. In all cases except the synthesis of EPN the two phase benzene/water system gave the best results. Acetone proved adequate in several cases, but lower reaction rates and lower yields were obtained. Since the coupling reaction is carried out in an aqueous system, there is opportunity for hydrolysis of both the phosphorochloridothionate and the products. The hydrolysis may become excessive if extended reaction times are required. Hydrolysis is a probable cause of the lower yield obtained in the synthesis of labelled diazinon, since the coupling reaction in this case was allowed to continue for an extended period of time. Attempted synthesis of EPN in the benzene/water solvent system resulted in lower yields due to hydrolysis, which led to the use of ethyl acetate as the solvent in this reaction. The excessive hydrolysis in this case is probably the result of the higher reactivity of phosphonothionates relative to phosphorothionates[19,20].

Two additional factors that influenced yields were the purity of the reagents used in the reactions and the recovery of the product absorbed to silver residues remaining in the reaction flask. Several extractions of the silver salts were sometimes necessary to recover the product. The yields of the reactions utilizing O,O-dimethyl phosphorochloridothionate were noticeably lower than reactions where the anlogous diethyl compound was used (Table 1). Contamination by phosphorothioic acids was not evident in reactions where the intermediate O,O-dialkyl phosphorochloridothionates were synthesized de novo.

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Table 1.

Synthesis of Pesticides Using the Silver Catalyzed Coupling Reaction

Compound	Reaction Time	Yield %	Reference for ir Spectrum
EPN	1 hr	92.7	21a Gunther, 1955, p. 663
Parathion	1/2 hr	89.1	21b Gunther, 1955, p. 660
Methyl Parathion	1/2 hr	78.9	21c Gunther, 1955, p. 667
O,O-Dimethyl O-(4-cyanophenyl) phosphorothionate	1 hr	67.7	22 Nishizawa, 1962
Sumithion	1/2 hr	69.0(97)[a]	23 Nishizawa, 1961
O,O-Diethyl O-(4-methylthiophenyl) phosphorothionate	1/2 hr	94.1	See text
Diazinon	Overnight	64.3(48.0)[b]	c

[a]After redistillation of the O,O-dimethyl phosphorochloridothionate.

[b]After purification on preparative thin-layer chromatography.

[c]Reference IR spectrum obtained from laboratory standard of diazinon.

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