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PREPARATION OF THE OPTICAL ISOMERS OF SOMAN  
FINAL REPORT

by

A. F. Fentiman, Jr.

February 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Pinacolyl alcohol was resolved into its enantiomers, which were reacted with methylchlorophosphine in such a way as to produce the corresponding O- pinacolyl methylphosphonothioic acids. These acids were resolved at phosphorus using the two enantiomers of <sup>alpha</sup> -phenethylamine. Approximately 10 grams of the following four compounds <sup>were</sup> prepared: the (-)- <sup>alpha</sup> -phenethylamine salt of C(+)-P(-)- and C(-)-P(-)-O-pinacolyl methylphosphonothioic acid and the (+)- <sup>alpha</sup> - phenethylamine salt of C(+)-P(+)- and C(-)-P(+)-O-pinacolyl methylphosphonothioic		

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acid. As a model for the preparation of all the optical isomers of soman, both the (+)-~~α~~phenethylamine salt and the sodium salt of C(+)~~P(+)~~-O-pinacolyl methylphosphonothioic acid were reacted with picryl fluoride in the presence and absence of alumina, which is postulated to be a fluoride-trapping agent. The best results were obtained using the sodium salt in the presence of alumina. In this case, the sodium salt of C(+)~~P(+)~~-O-pinacolyl methylphosphonothioic acid was converted in essentially quantitative yield to a mixture consisting of 76% C(+)~~P(-)~~-soman contaminated with 24% C(+)~~P(+)~~-soman. It is conceivable that the isomers of soman could be prepared in even greater optical purity and that this approach could lead to a method for the preparation of milligram to gram quantities of all four of the optical isomers of soman.

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SUMMARY

The purpose of this work was to prepare gram quantities of the four optical isomers of soman (O-pinacolyl methylphosphonofluoridate, or GD). This was to be accomplished in the following way:

- Resolve pinacolyl alcohol into its enantiomers
- Prepare the corresponding O-pinacolyl methylphosphonothioic acid from each enantiomer
- Resolve each O-pinacolyl methylphosphonothioic acid at phosphorus
- React each of the four completely resolved acids or their salts with picryl fluoride
- Isolate and characterize the optical isomers of soman.

A quantity of each of the two enantiomers of pinacolyl alcohol was prepared. The (+)-isomer was prepared using the classical method involving fractional crystallization of the (-)-brucine salt of hydrogen pinacolyl phthalate and subsequent hydrolysis of the resolved ester. The (-)-isomer was prepared by a previously unreported method involving the separation of the diastereomeric esters formed by reacting racemic pinacolyl alcohol with D-alanine.

Each of the pinacolyl alcohol enantiomers was then reacted with methylchlorophosphine (followed by treatment with sodium bicarbonate and then sulfur) to form the corresponding O-pinacolyl methylphosphonothioic acid. These acids were resolved at phosphorus using the two enantiomers of  $\alpha$ -phenethylamine. At least 10 grams of the following four compounds was prepared: the (-)- $\alpha$ -phenethylamine salt of C(+)-P(-)- and C(-)-P(-)-O-pinacolyl methylphosphonothioic acid and the (+)- $\alpha$ -phenethylamine salt of C(+)-P(+)- and C(-)-P(+)-O-pinacolyl methylphosphonothioic acid.

Using either the (+)- $\alpha$ -phenethylamine salt or the sodium salt of C(+)-P(+)-O-pinacolyl methylphosphonothioic acid, reactions with picryl fluoride were carried out in an attempt to establish conditions for preparing gram quantities of the optical isomers of soman. In the presence of alumina, the sodium salt was converted in essentially quantitative yield to a mixture consisting of 76% C(+)-P(-)-soman and 24% C(+)-P(+)-soman. It is postulated that alumina acts as a fluoride-trapping agent which retards the racemization of the soman isomer after it is generated. In the absence of alumina, almost complete racemization occurs at phosphorus.

With additional work involving the investigation of this and other fluoride scavengers, it is conceivable that the optical purity of the isomers of soman could be increased and that this approach could lead to a method for producing the desired quantities of the soman isomers. A method for the chromatographic purification of the isomers is also discussed.

FOREWORD

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## STATEMENT OF THE PROBLEM

Soman (O-pinacolyl methylphosphonofluoridate, or GD) exists in four isomeric forms arising from the presence of two centers of asymmetry in the molecule. Each of these isomers has a different biological activity in terms of both toxicology and response to certain enzymes. In order to study these isomers in various biological systems, a quantity of each of the pure isomers is needed. The goal of this study is to establish a chemical method for producing gram quantities of each of the four optical isomers of soman in pure form.

### Background

The four optical isomers of soman exist because of the presence of an asymmetric center in the pinacolyl moiety and at the phosphorus atom. In this report we will use the designation of H. P. Benschop et al. to identify the four isomers of soman.(1) The stereoisomers are designated C(+P(+), C(+P(-), C(-P(-), and C(-P(+), in which C stands for the chiral center in the pinacolyl moiety and indicates whether the soman is derived from (+)-pinacolyl alcohol or from (-)-pinacolyl alcohol. The absolute configuration of (+)-pinacolyl alcohol is (S) by the Cahn-Ingold-Prelog convention as determined by J. Jacobus, Z. Majerski, K. Mislow and Von R. Schleyer.(2) Of course, the (-)-isomer has the (R) configuration. Although the absolute configuration at phosphorus in soman has not been formally established, by analogy with the work on sarin in which the absolute configuration at phosphorus is (S) for the (-) or levorotatory isomer, it is concluded that the P(-) is (S) for soman and for the corresponding alkyl methylphosphonothioic acids.(3-6) This allows us to tentatively designate the absolute configuration for each of the isomers. Thus, for soman, C(+P(+)) is  $S_C R_P$  in which  $S_C$  is the Cahn-Ingold-Prelog designation of (S) for the absolute stereochemistry at carbon and  $R_P$  is the Cahn-Ingold-Prelog designation of (R) for the absolute stereochemistry at phosphorus. Then C(+P(-)) is  $S_C S_P$ , C(-P(+)) is  $R_C R_P$ , and C(-P(-)) is  $R_C S_P$ .

In the study by Benschop, (1) it was shown that C(-)P(-)-soman is the most toxic of the isomers in terms of LD<sub>50</sub> value (SC, mice). It is about 130 times as toxic as the least toxic isomer, which is C(+P(+)-soman, and about 4 times as toxic as racemic soman. The bimolecular reaction rates for the inhibition of acetylcholinesterase from electric eel shown that C(+P(-)- and C(-)P(-)-soman inhibit at a rate about 10<sup>4</sup> times greater than that of the other stereoisomers, C(+P(+)- and C(-)P(+)-soman.(1) Earlier studies also indicate that the toxicological properties of the soman isomers can differ significantly,(7) and that they have widely different rates of reaction with acetylcholinesterase.(8) Variations are also observed in the response of inhibited enzyme to reactivating agents and in the rate of detoxification of enzymes in blood, liver and skin.(9,10) In view of the observed and potential

differences in the toxicological properties of the soman isomers, researchers studying anti-cholinesterase agents would benefit from the availability of preparative quantities of the pure isomers.

There are three approaches to the isolation of the stereoisomers of anticholinesterase agents. One approach is to use preparative chromatography in conjunction with a chiral substrate to separate the isomers. Attempts were made by H. P. Benschop and his coworkers to isolate the four isomers of soman using capillary gas-liquid chromatography (GLC).(1,11) They concluded that this procedure did not allow isolation of the stereoisomers in quantities that were experimentally useful. We will have more to say about this approach later.

A second approach that was employed successfully by Benschop et al. (1) uses enzymes to stereospecifically hydrolyze one or the other of the soman isomers, leaving behind the unreacted, essentially pure, isomer of the opposite configuration. These hydrolyses were done on isomers in which the asymmetric carbon of the pinacolyl moiety had already been resolved. The major disadvantage of this approach is that the usual scale for preparing any of the isomers is 0.1 to 2 mg. This is sufficient for the valuable experiments of Benschop and coworkers but it would be difficult to supply other researchers from these preparations.

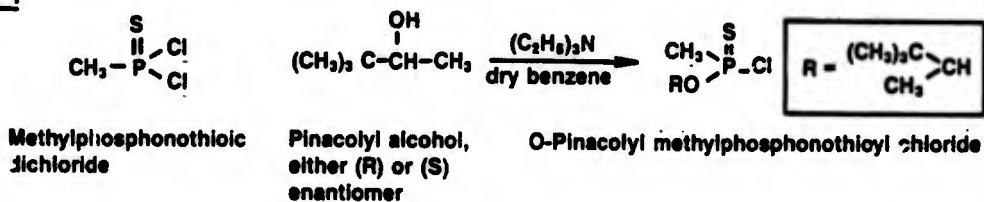
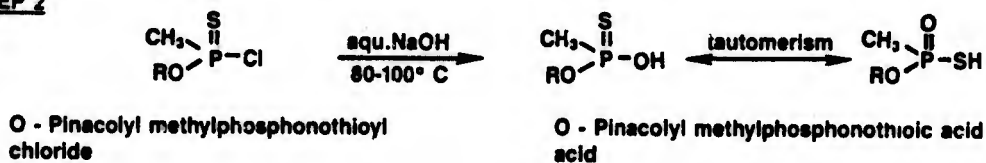
A third approach is exemplified by the work of Boter et al. in which the two optical isomers of isopropyl methylphosphonofluoridate (sarin) were prepared from optically pure precursors.(12) It is primarily on this work that the current program is based. Surprisingly, no one has previously attempted to prepare the isomers of soman using this approach.

### APPROACH TO THE PROBLEM

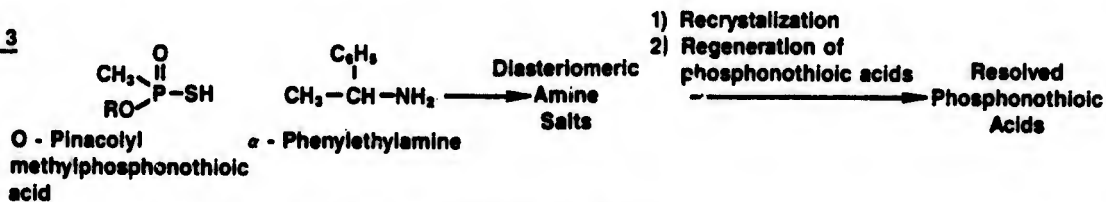
Figure 1 shows the proposed scheme for the preparation of optically active soman isomers. This is essentially the method used by Boter et al. (12), using one or the other of the enantiomers of pinacolyl alcohol in place of isopropyl alcohol.

The first task in this program was to prepare a quantity of each enantiomer of pinacolyl alcohol. Step 1 of Figure 1 shows the reaction of pinacolyl alcohol with methylphosphonothioic dichloride to produce O-pinacolyl methylphosphonochloridothioate. This is based on the work of Hoffmann et al. for the preparation of other O-alkyl esters.(13) The intention was then to hydrolyze the chloridothioate to the corresponding O-pinacolyl methylphosphonothioic acid, as Hoffmann et al. did with other O-alkyl derivatives.(14) Unfortunately, we were unable to prepare O-pinacolyl methylphosphonothioic acid by this method, so an alternative method was used.

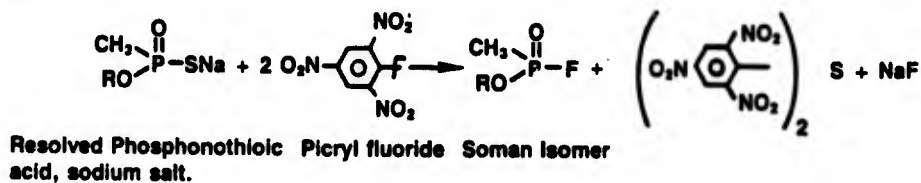
Resolution of O-pinacolyl methylphosphonothioic acid at phosphorus was to be carried out using the enantiomers of  $\alpha$ -phenethylamine

**STEP 1****STEP 2**

Two reaction mixtures should be in hand at the conclusion of Step 2:

**STEP 3**

Four resolved products should be in hand at the conclusion of Step 3:

**STEP 4**

Four optically active products should be in hand at the conclusion of Step 4.

FIGURE 1. PROPOSED SCHEME FOR THE PREPARATION OF OPTICALLY ACTIVE SOMAN ISOMERS

(Figure 1, Step 3). This methodology was worked out for the resolution of various O-alkyl methylphosphonothioic acids by Boter and Platenburg.(15) It was equally successful in our hands.

After resolution, one of the salts of the O-pinacolyl methylphosphonothioic acid was to be reacted with picryl fluoride to prepare the corresponding soman isomer (Figure 1, Step 4). This brings us back to the work of Boter et al. (12), who established picryl fluoride as a reagent for preparing various phosphonofluoridates (16,17) based on previous work with 2,4-dinitrofluorobenzene by others. (18,19) The preparation of the optical isomers of soman was designed to obtain isomers of high optical purity. Those strategies were tried and others will be discussed.

## RESULTS AND DISCUSSION

This section is divided into six parts. The following subjects are discussed and results presented for each subject: resolution of pinacolyl alcohol, preparation of O-pinacolyl methylphosphonothioic acid, resolution of O-pinacolyl methylphosphonothioic acid, chromatography of soman isomers, mechanism of the conversion of O-pinacolyl methylphosphonothioic acid derivatives to soman isomers and strategies to prevent racemization, and conversion of the salts of C(+)-P(+)-O-pinacolyl methylphosphonothioic acid into an optically active isomer of soman.

### Resolution of Pinacolyl Alcohol

Racemic pinacolyl alcohol was resolved in two ways. The (+)(S)-pinacolyl alcohol was prepared using the classical method involving fractional crystallization of the (-)-brucine salt of hydrogen pinacolyl phthalate and subsequent hydrolysis of the resolved ester. The specific procedure employed is based on that described in Organic Reactions (20) for resolution of 2-octanol and on the procedure described by Pickard and Kenyon (21) for the resolution of pinacolyl alcohol.

Pinacolyl hydrogen phthalate was prepared in approximately 75% yield by reacting pinacolyl alcohol and phthalic acid. The melting point (mp) of the crystallized material was 85<sup>o</sup>-86<sup>o</sup>C, identical to the literature value.(21) The half-ester was reacted with an equimolar quantity of (-)-brucine in acetone. The (-)-brucine salt of the half-phthalate of (+)-pinacolyl alcohol melted at 148.5<sup>o</sup>-150<sup>o</sup>C with  $[\alpha]_D^{20} -4.16^{\circ}$  (C=5, ethanol). The corresponding literature values (21) are 148<sup>o</sup>-151<sup>o</sup>C and -4.22<sup>o</sup>. The (+)-pinacolyl monophthalate was liberated from the salt, crystallized and then hydrolyzed to (+)-pinacolyl alcohol, which was distilled. In this way, approximately 40 g of high-quality (+)-pinacolyl alcohol was prepared. The isolation of (-)-pinacolyl alcohol in pure form as a by-product in the preparation of (+)-pinacolyl alcohol is difficult.



40% yield and was characterized by infrared and NMR spectroscopy and by formation of the dicyclohexylamine salt which melted at 171<sup>o</sup>-172<sup>o</sup>C [literature value: 170<sup>o</sup>-172<sup>o</sup>C (23)].

Next, racemic pinacolyl alcohol was reacted with an equivalent amount of methylchlorophosphine in benzene and the reaction mixture treated as before. In this way, O-pinacolyl methylphosphonothioic acid was prepared in 48% yield. The infrared spectrum and the NMR spectrum (Figure 2) were consistent. The dicyclohexylamine salt was prepared and had mp 187<sup>o</sup>-188<sup>o</sup>C after crystallization from acetone.

Finally, the reaction was carried out using the resolved pinacolyl alcohol. Using 5.0 g of (-)-pinacolyl alcohol, 5.7 g (59% yield) of C(-)-O-pinacolyl methylphosphonothioic acid was prepared. This material was characterized by IR (Figure 3), NMR (Figure 4) and mass spectrometry (Figure 5). The mass spectrum was determined under chemical ionizing (CI) conditions using methane/ammonia as ionizing reagent. The ions are interpreted in the figure. Abundant proton and ammonium adduct ions are formed and each fragments by eliminating the pinacolyl group as an olefin. The proton NMR spectrum shows the protons of the tert.-butyl group at  $\delta$ 0.89, a doublet for the other methyl group of the pinacolyl group centered at  $\delta$ 1.26, and a doublet for the protons of the methyl group attached to phosphorus centered at  $\delta$ 1.83 with the large splitting associated with phosphorus coupling. The single proton of the pinacolyl group is split by the methyl group and the phosphorus atom to give a multiplet centered at  $\delta$ 4.41. The spectra are all consistent. When working on a larger scale, even higher yields were obtained, on the order of 70%, with identical spectra for these batches. Experimental details are presented in the Experimental Procedures section.

The same reactions were carried out with (+)-pinacolyl alcohol. The yields were on the same order (65 to 70%) and the spectra were identical to those obtained when (-)-pinacolyl alcohol was used, so they will not be reproduced here.

With a supply of both C(-)- and C(+)-O-pinacolyl methylphosphonothioic acid in hand, the next step was to carry out the resolution at phosphorus.

#### Resolution of O-Pinacolyl Methylphosphonothioic Acid

The resolution at phosphorus is based on the work of Boter and Platenburg.(15) The clear presentation of experimental detail by Moriyama and Bentrude(6) for the resolution of O-isopropyl methylphosphonothioic acid made this work much easier.

The C(-)-acid was resolved into the C(-)P(-)-acid and the C(-)P(+)-acid, using first (-)- $\alpha$ -phenethylamine and then (+)- $\alpha$ -phenethylamine. For example, C(-)-acid was treated with one-half equivalent of (-)- $\alpha$ -phenethylamine in ether. The less soluble salt, which we designate

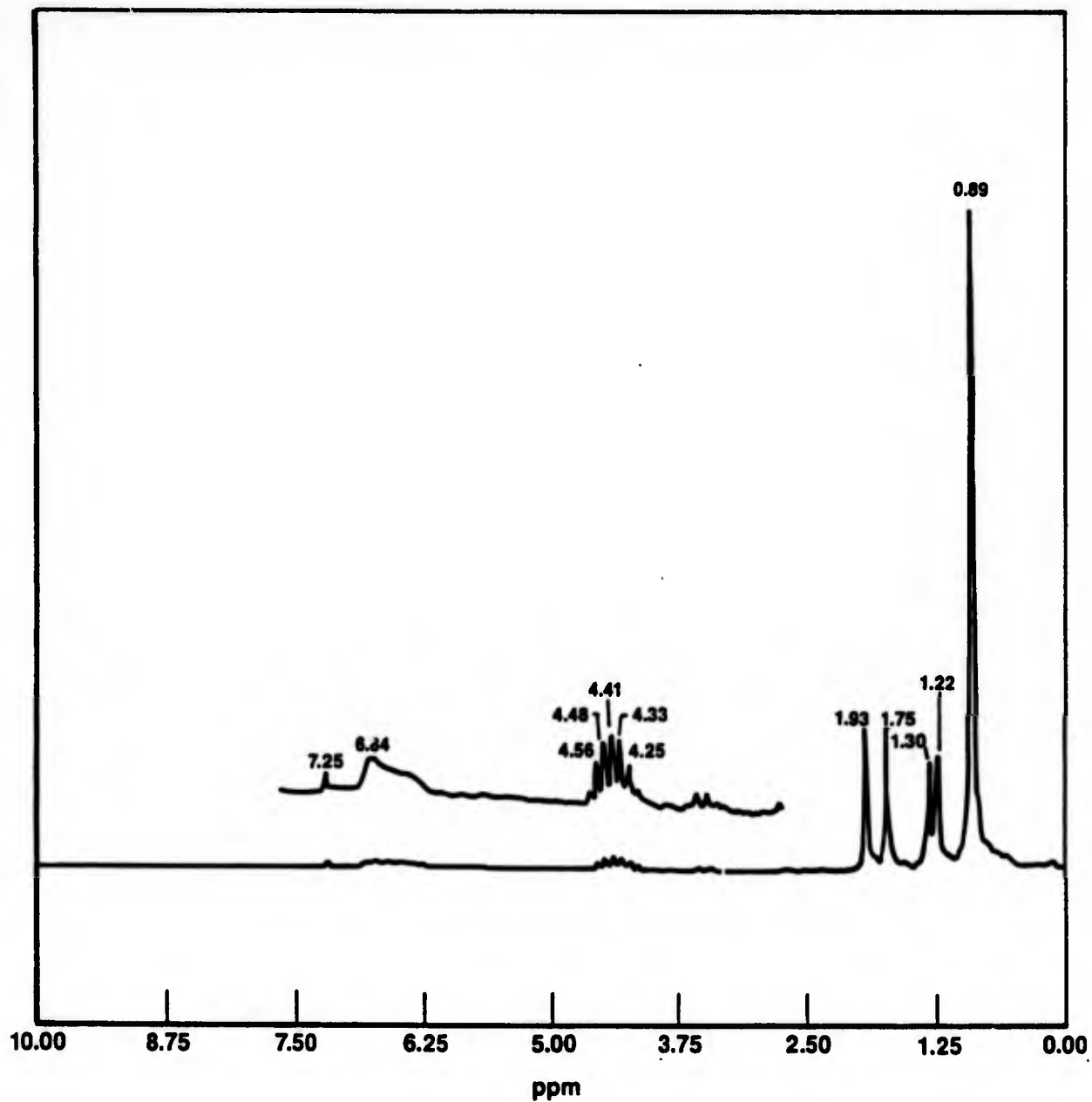


FIGURE 2.  $^1\text{H-NMR}$  SPECTRUM OF O-PINACOLYL METHYLPHOSPHONOTHIOIC ACID

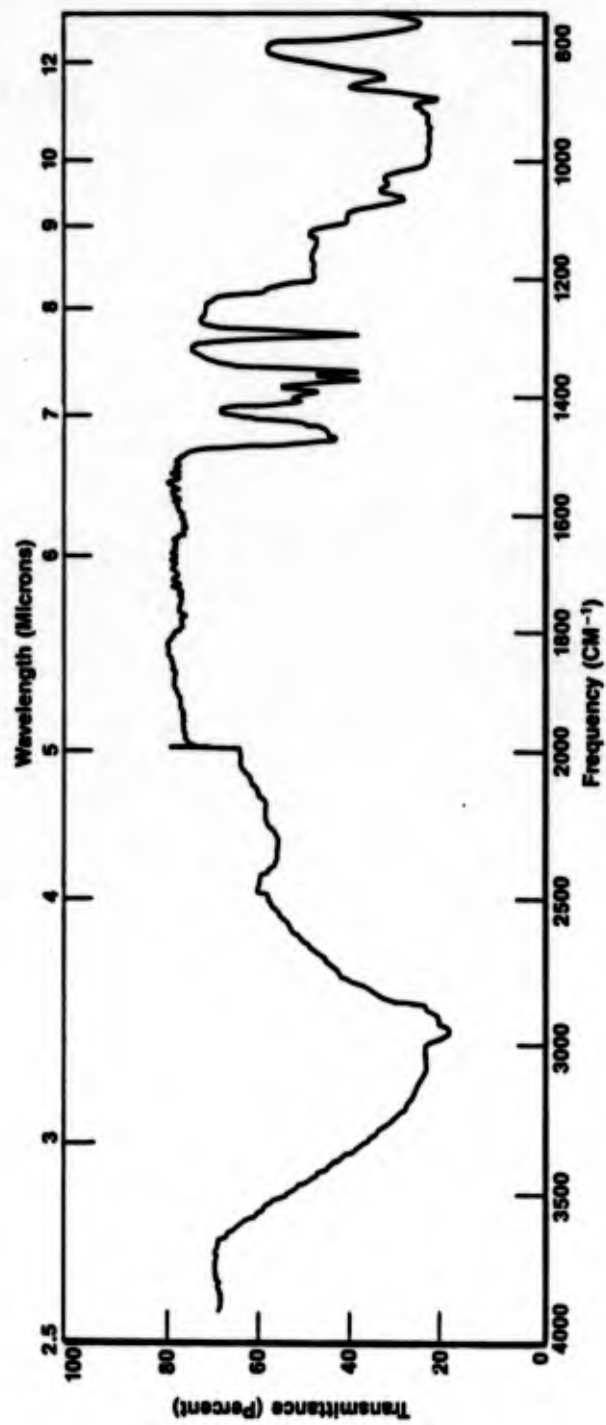


FIGURE 3. INFRARED SPECTRUM OF C(-)-O-PINACOLYL METHYLPHOSPHONOTHIOIC ACID

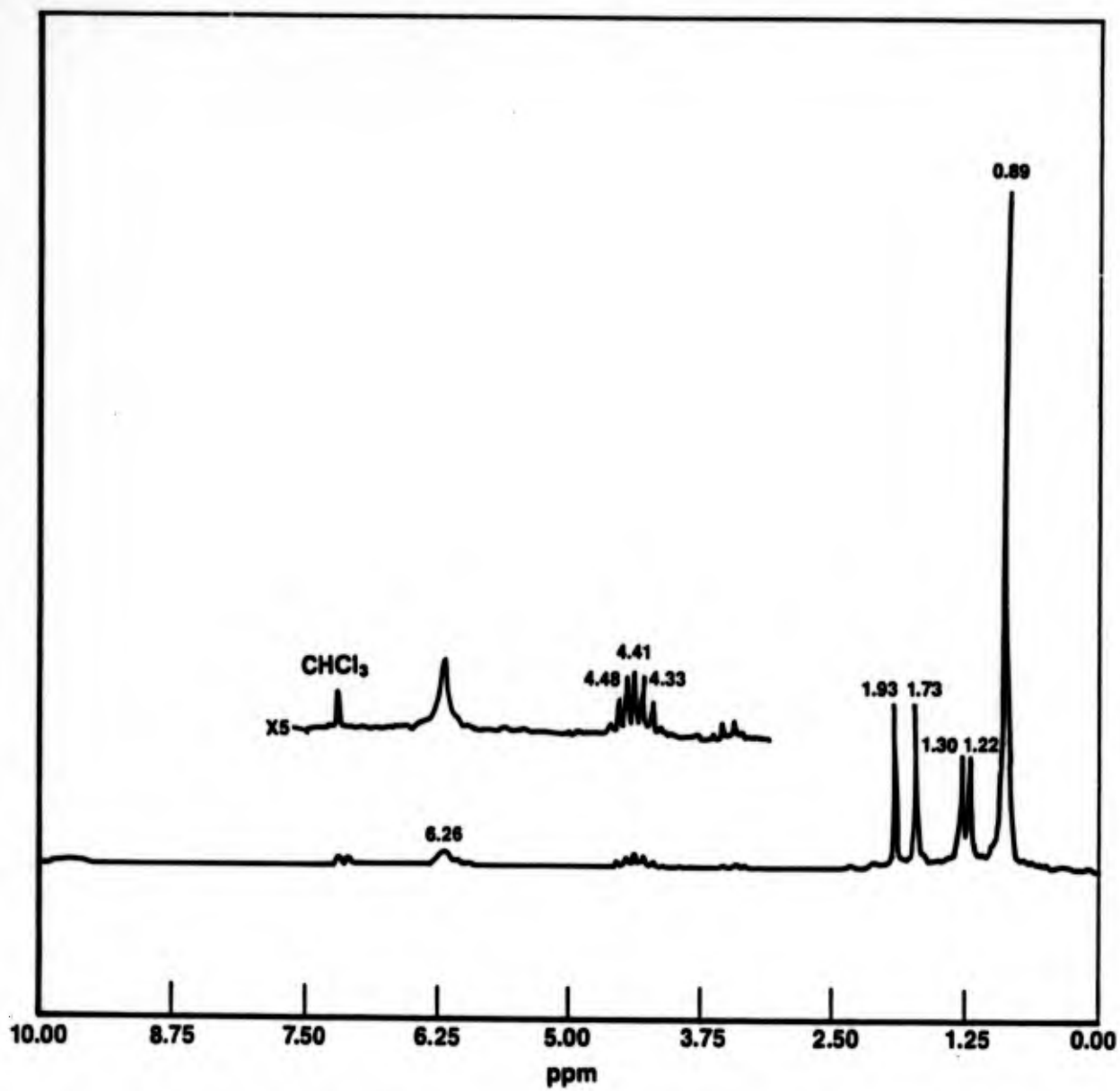


FIGURE 4.  $^1\text{H-NMR}$  SPECTRUM OF C(-)-O-PINACOLYL METHYLPHOSPHONOTHIOIC ACID

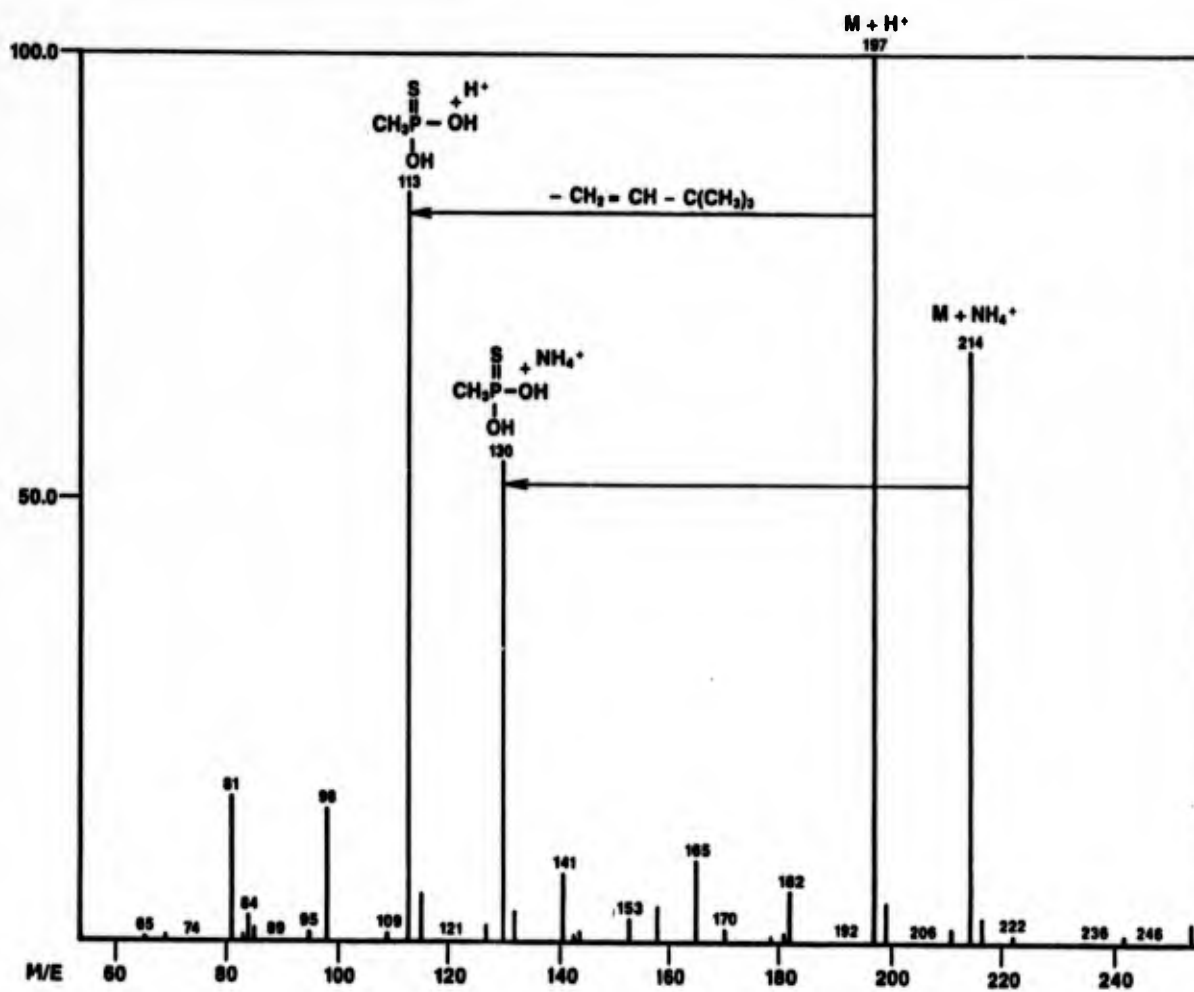
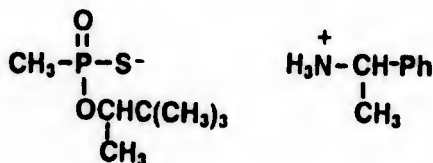


FIGURE 5. CI MASS SPECTRUM OF (-)-O-PINACOLYL METHYLPHOSPHONOTHIOIC ACID

C(-)P(-),A(-), precipitates and was purified by crystallization from ethyl acetate. Our designation A(-) refers to the fact that the salt results from the addition of (-)-amine. The original mother liquor containing C(-)P(+)-acid was treated with acid to free it from any (-)- $\alpha$ -phenethylamine and then the liberated C(-)P(+)-acid was reacted with (+)- $\alpha$ -phenethylamine to give the C(-)P(+),A(+)-salt. This was also purified by crystallization. Thus from C(-)-O-pinacolyl methylphosphonothioic acid we prepared the two salts C(-)P(-),A(-) and C(-)P(+),A(+).

Using C(+)-O-pinacolyl methylphosphonothioic acid, the other two resolved salts were prepared. These we designated C(+P(+),A(+)) and C(+P(-),A(-)). Experimental details are presented later. Table 1 summarizes the data for these resolved salts, the general structure of which is shown below.



### Chromatography of Soman Isomers

In order to monitor the conversion of the optical isomers of O-pinacolyl methylphosphonothioic acid to the optical isomers of soman, it is desirable to have a method that will detect and quantify all of the soman isomers. Such a method has been developed by Benschop and his coworkers.(1,11) The separation is based on a capillary gas chromatography (GC) method using a conventional column in conjunction with a chiral column. A combination Carbowax/Chirasil-Val III column was used. However, with the Chirasil-Val III column obtained from our supplier, resolution of the isomers was never achieved. Several attempts to optimize the column conditions were made, but separation of each isomer in a mixture was not greater than 25% peak to peak from baseline. It was possible for us to completely separate the diastereomeric pairs of the isomers using a more conventional column. A Durabond-S (DB-5) column gave baseline resolution of the C(-)P(+), C(+P(-) pair from the C(+P(+), C(-)P(-) pair. This is shown in Figure 6. The earlier peak is the C(-)P(+), C(+P(-) pair, based on a comparison with the separations of Benschop et al.(11)

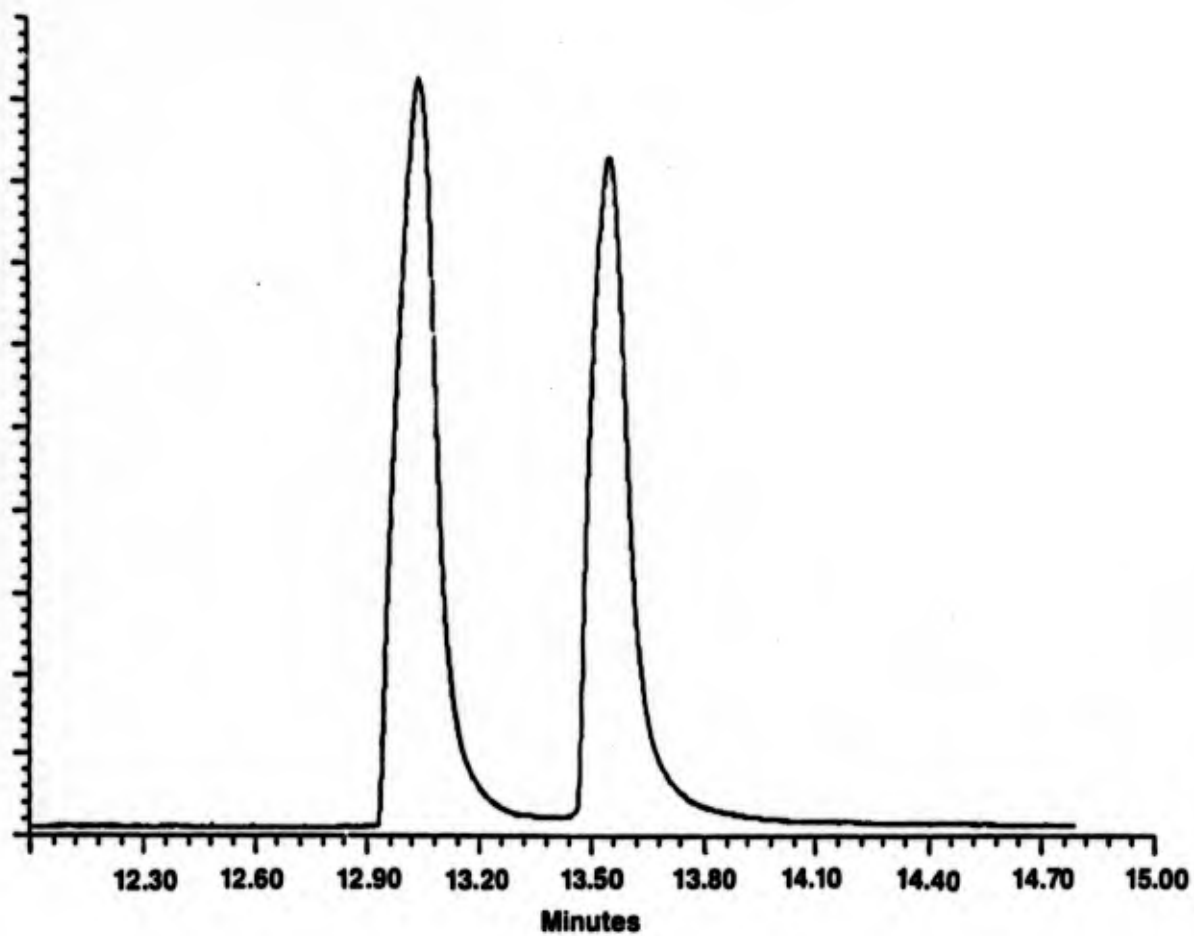
Since the diastereomers of soman can be separated, it is still possible to monitor the formation of soman isomers, provided that the carbon atom does not racemize and that it is of one configuration to begin with. Thus, C(+P(-) will be resolved with respect to C(+P(+), and

TABLE 1. PHYSICAL, ANALYTICAL, AND ANTICHOLINESTERASE ACTIVITY DATA FOR THE  $\alpha$ -PHENETHYLAMINE SALTS OF RESOLVED O-PINACOLYL METHYLPHOSPHONOTHIOIC ACIDS

Structure Designation	Melting Point, °C	[ $\alpha$ ] <sub>D</sub> (C-6, MeOH)	Anticholinesterase Activity(a) Soman Equivalents, ng/ml	Elemental Analyses Found, % (b)			
				C	H	N	S
C(-)P(-), A(-)	190-191	-14.8	6.2	57.04	9.04	4.43	10.16
C(-)P(+), A(+)	173.5-174.5	+2.7	4.7	56.94	8.91	4.39	10.28
C(+ )P(+), A(+)	190-191	+14.6	3.8	57.04	8.78	4.39	9.93
C(+ )P(-), A(-)	173.5-174.5	-2.9	6.3	57.02	9.16	4.37	10.04

(a) Determinations were made using a colorimetric method based on the work of Ellman et al. (24). A Technicon Autoanalyzer was used for addition of reagents, incubation and colorimetric readings. Solutions of each of the salts at a concentration of 1.0 mg/ml were analyzed and the enzyme employed was acetylcholinesterase from electric eel, obtained from Sigma Chemical Company.

(b) Calculated for C<sub>15</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>PS, %: C, 56.76, H, 8.89; N, 4.41; P 9.76; and S, 10.10.



**FIGURE 6. SOMAN SEPARATION USING DB-5 COLUMN**

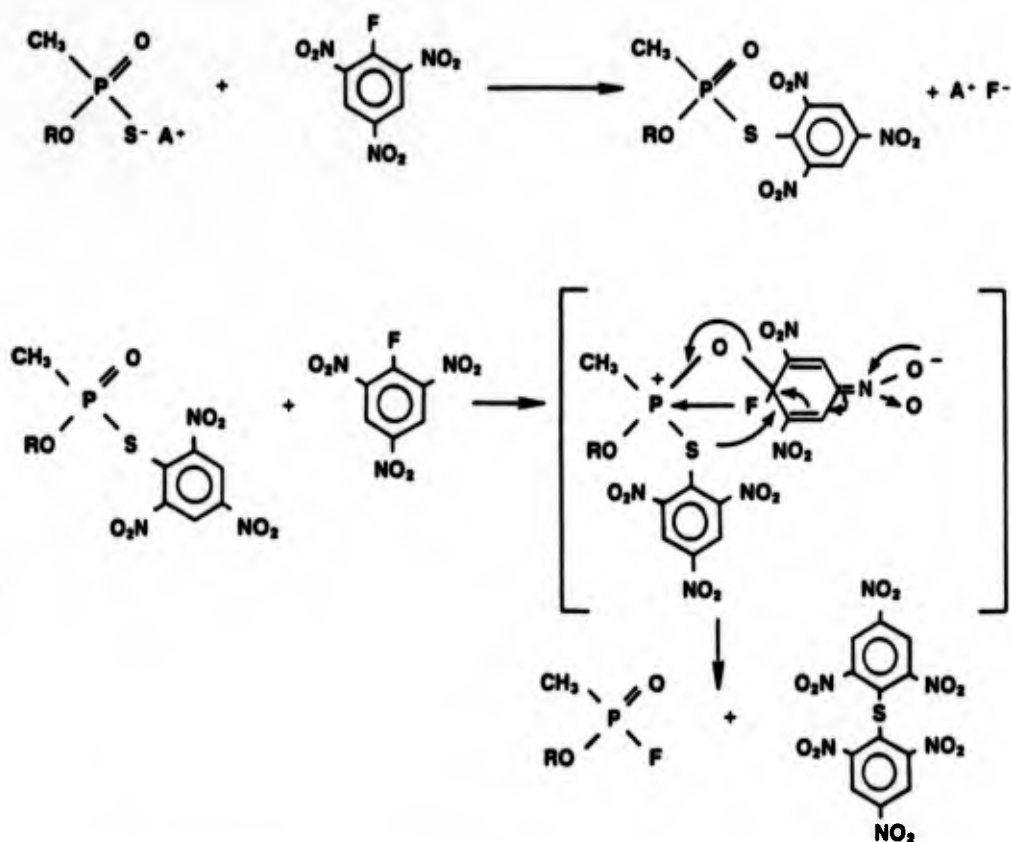
C(-)P(+) with respect to C(-)P(-). For example, in monitoring the conversion of C(+)P(+)-O-pinacolyl methylphosphonothioic acid to C(+)P(-)-soman, only one peak would be expected if the product were optically pure. However, any racemization at phosphorus would produce C(+)P(+)-soman, which would show up as a second peak. We proceeded under these assumption and our results indicate that they were probably correct.

The flame ionization detection (FID), (GC) conditions are listed below:

GC Model	Carlo Erba 2150
GC Column	J&W DB-5 column 30 long x .22 m inside diameter x .25 $\mu$ m film thickness fused silica
Detector	Flame ionization detector
Carrier Gas	Hydrogen
Carrier Gas Velocity	50 cm/sec
Injector Temperature	250°C
Detector Temperature	250°C
Oven Temperature	80°C isothermal
Injection Mode	Split 20:1 ratio
Injection Volume	5 $\mu$ L
Integrator	Shimadzu C-RIA Chromatopac

#### Mechanism of the Conversion of O-Pinacolyl Methylphosphonothioic Acid Derivatives to Soman Isomers and Strategies to Prevent Racemization

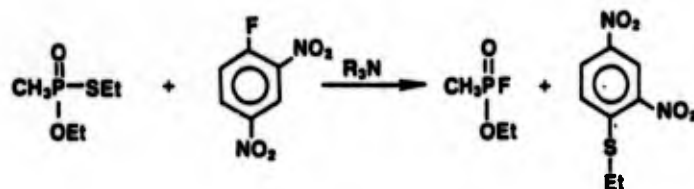
The key to this entire program is the reaction of optically pure O-pinacolyl methylphosphonothioic acid with picryl fluoride in such a way that optically pure isomers of soman result and do not racemize. To achieve this, it is necessary to understand the mechanism of the reaction and to devise strategies to prevent racemization. Boter and Van Den Berg have discussed the mechanism of this reaction.(16) They picture the reaction taking place in two stages as shown below.



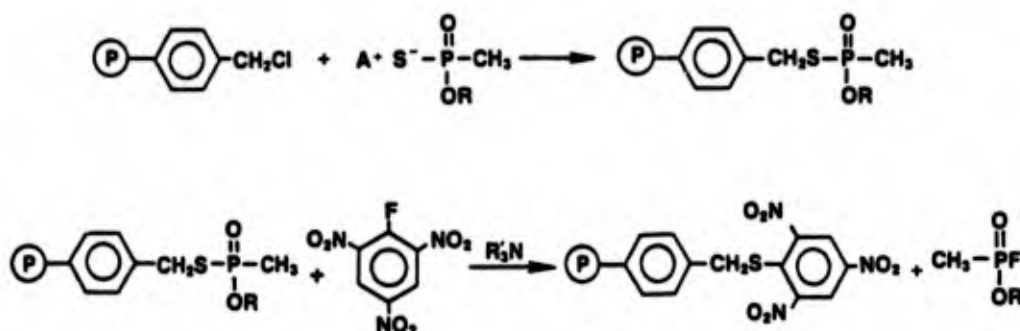
The following observations were presented in support of this mechanism: (a) 2 moles of picryl fluoride are required; (b) the reaction proceeds with inversion of configuration at phosphorus; and (c) by polarimetry, it appears that a fast first step occurs, followed by a slow second step or steps.

Although the complex may not exist as drawn in the second stage because there is no need to postulate a completely concerted reaction, the basic facts seem to be well accounted for by this mechanism. Also, picryl sulfide has been isolated in high yield in these reactions. It should further be noted that the presence of fluoride ion leads to racemization of soman isomers (1,12), as does moisture or water. An additional observation made by Boter and Van Den Berg is that using the non-hygroscopic dicyclohexylamine salt of optically active methylphosphonothioic acids gave only racemic products, whereas the deliquescent sodium salts gave optically active products. The reasons for this have not been explained. Perhaps the counter ion plays a role in tying up the fluoride ion. The ammonium ion is a relatively soft acid, while the fluoride ion can be considered a very hard base. This would lead to a relatively unstable ion pair with fluoride available to react. Sodium ion and fluoride form a more stable ion pair. Perhaps using lithium would even more completely neutralize the fluoride ion and thus hinder even more the racemizing action of the fluoride ion.

In a separate study, Bebbington and Ley(19) carried out the following reaction:



A 79% yield was obtained using equivalent amounts of ester and fluorinating agent. However, when optically active phosphonothioates were used, racemization occurred. The possibility of using the more reactive picryl fluoride to accomplish this conversion in a stereospecific way was considered. The advantage of such a method would be to eliminate the fluoride ion produced in the first step when the reaction is carried out in the usual way. We also considered forming a polymer-bound ester so that the by-product sulfide would be retained on the polymer and distillation might not be necessary. The reactions are shown below where P represent a polymer substrate such as Merrifield resin.



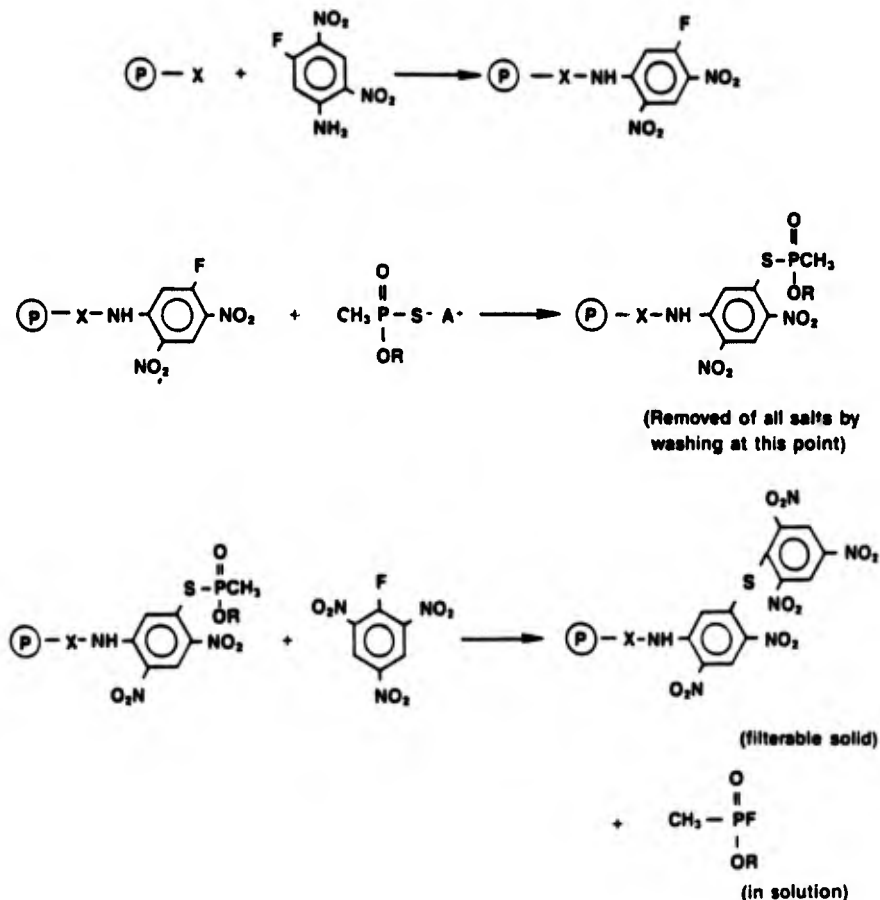
As a model, we reacted the (+)- $\alpha$ -phenethylamine salt of C(+)-P(+)-O-pinacolyl methylphosphonothioic acid with benzyl chloride to produce, in quantitative yield, the S-benzyl ester. The product was not

distilled but appeared to be reasonably pure by NMR (Figure 7). The rotation of this material was  $+6.5^\circ$  (C=6, methanol). However, attempts to react this compound with picryl fluoride in methyl acetate in the presence of triethylamine gave no detectable soman. Since the model compound did not give encouraging results, the polymer approach was abandoned.

Other methods were considered that might reduce the amount of fluoride ion present in the reaction mixture. These include the use of fluoride ion-trapping agents, ion exchange resins, phase catalysis and the use of other polymer-bound reagents.

Ion exchange resins did not offer much promise since, in most of these resins, fluoride ion is one of the least easily retained, and the exchange ion may be an equally serious problem. Phase catalysis would be possible if an unreactive pair of solvents could be identified. A method that offers some promise is shown below. This was not pursued because of time limitations and the rather complex chemistry involved.

Since 2,4-dinitro-5-fluoroaniline is commercially available, it may be possible to create a solid-phase derivative in such a way that the fluoride ion of the first reaction is removed before the reaction with picryl fluoride to produce soman is carried out. This sequence is worth consideration:



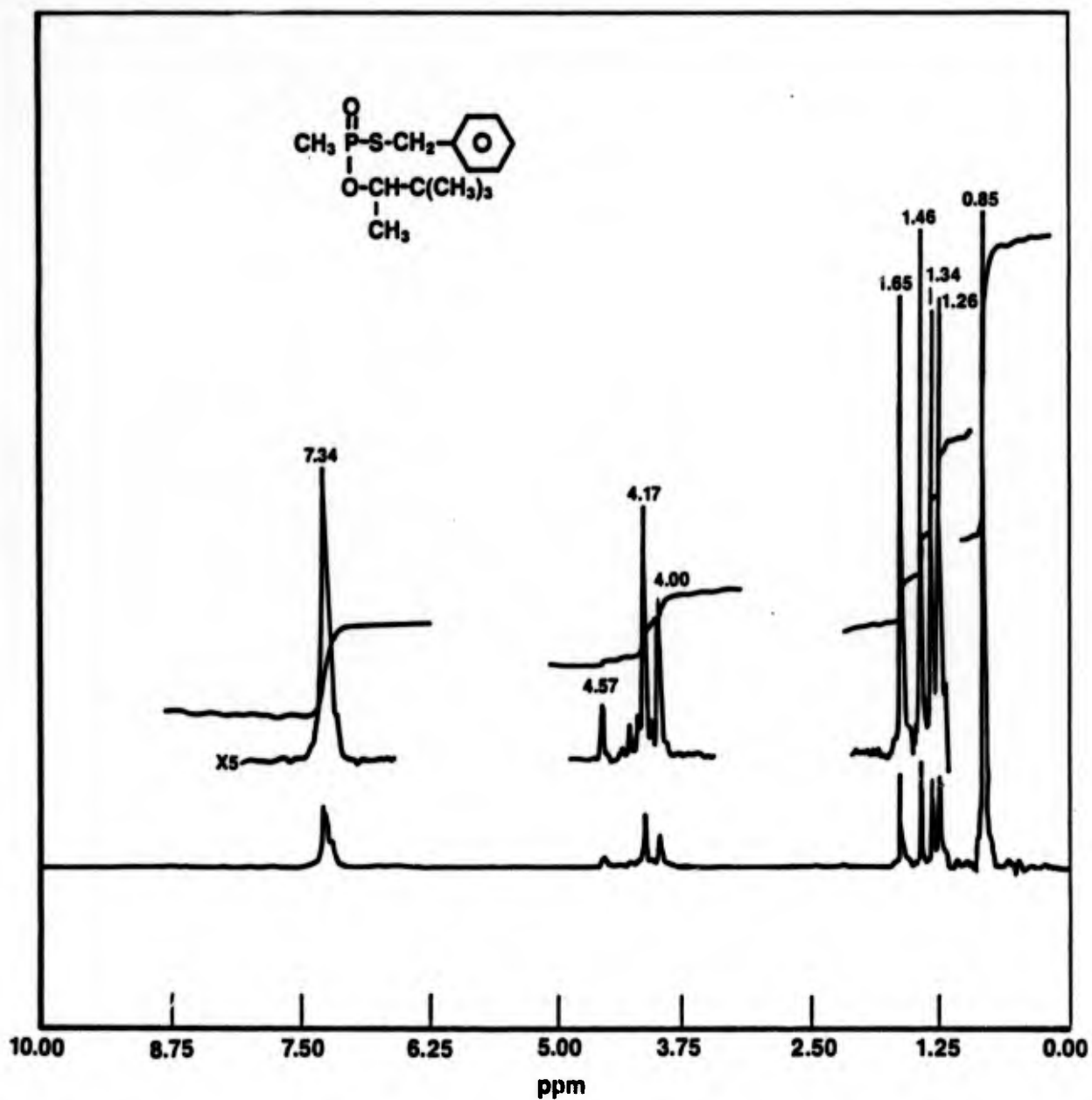


FIGURE 7. <sup>1</sup>H-NMR SPECTRUM OF C(+)-P(+)-O-PINACOLYL S-BENZYL METHYLPHOSPHONOTHIOIC ACID

This leaves only fluoride-trapping agents as a real possibility for facilitating the formation of optically pure soman isomers at this time. Fluoride ion has a high affinity for certain absorbents, such as alumina or silica gel. To remove small amounts of fluoride ion from water during analytical procedures, the water is passed through a bed of alumina. Thus, even in a deactivated form, alumina will retain fluoride ion. We reasoned that alumina in the reaction mixture for the preparation of soman isomers might remove the fluoride ion produced in the first step and prevent it from racemizing the soman isomer produced in the second step. Activated alumina might also act as an internal drying agent and thus prevent hydrolysis of the soman isomer. This would further prevent the formation of fluoride ion which occurs with hydrolysis.

In the next section, four experiments are described in which we attempted to convert C(+)-P(+)-O-pinacolyl methylphosphonothioic acid salts to C(+)-P(-)-soman.

#### Conversion of the Salts of C(+)-P(+)-O-Pinacolyl Methylphosphonothioic Acid Into an Optically Active Isomer of Soman

Soman is an extremely toxic substance. All of the experiments that might lead to the generation of soman were carried out at the Hazardous Materials Laboratory in accordance with a Standard Operating Procedure established with the U.S. Army Medical Research Institute of Chemical Defense.

Four experiments were carried out. In each case, a salt of C(+)-P(+)-O-pinacolyl methylphosphonothioic acid was reacted with two equivalents of picryl fluoride. In two experiments the (+)- $\alpha$ -phenethylamine salt was used and in the other two experiments the sodium salt was used. In one experiment for each of the salts, alumina was present in the reaction mixture. The reactions were carried out as follows: The reactants were placed in a round-bottomed flask and methyl acetate was added all at once. The reaction mixture was stirred for 30 minutes and then the solvent and product were distilled into a receiver at dry ice temperature. The final pressure was 0.2 mm Hg. Two fractions were collected, the first when the reaction flask was maintained at ambient temperature with a water bath, and the second when the water bath was heated to 45<sup>o</sup>-50<sup>o</sup>C. This was necessary in order to recover all of the soman produced. Small differences in the optical purity of the soman from fraction 1 and fraction 2 were observed. Distillation at pressures down to 0.05 mm Hg at ambient temperature would be desirable, but our system would not achieve that pressure because of the nature of the pump and the number of traps involved.

Each fraction was then analyzed by GLC as described in the section on chromatography. Figure 8 shows a gas chromatogram of laboratory-grade soman as received from the Medical Research and

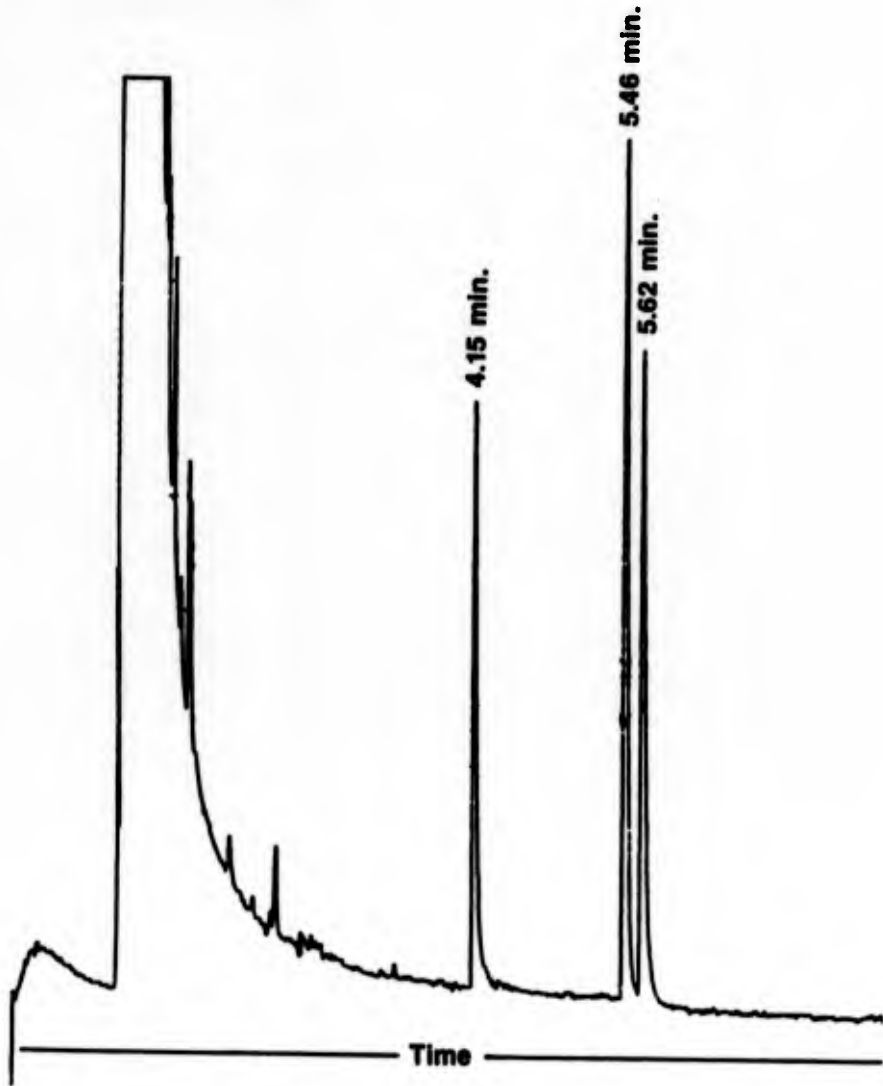


FIGURE 8. GAS CHROMATOGRAM OF RACEMIC SOMAN

Development Command. In order to calculate the relative amounts of C(+)-P(-)- and C(+)-P(+)-soman produced in our experiments, the following formula was used:

$$\frac{W[C(+)-P(-)]}{W[C(+)-P(+)]} = \frac{A[C(+)-P(-)]}{A[C(+)-P(+)]}$$

where W[C(+)-P(-)] is the weight of the diastereomer in the first peak (retention time, about 5.4 minutes) and W[C(+)-P(+)] is the weight of the diastereomer in the second peak (retention time, about 5.6 minutes) with A[C(+)-P(-)] and A[C(+)-P(+)] the corresponding areas of the peaks. From this ratio the relative amounts of the two diastereomeric isomers can be calculated from the areas of the peaks produced by the products.

Table 2 summarizes the reactions carried out in attempts to produce an optical isomer of soman. The sodium salts were prepared by liberating the acid from the amine salt and then reacting it with sodium hydride. They were then dried under vacuum to a constant weight. The details are given in Experimental Procedures. The picryl fluoride was prepared by the method of Shaw and Seaton.(25) After recrystallizing the picryl fluoride from carbon tetrachloride, it had an mp of 131<sup>o</sup>-133<sup>o</sup>C, in good agreement with that reported for the higher melting allotropic form, which is 131<sup>o</sup>-132<sup>o</sup>C.(24) The neutral alumina used was supplied by Fisher Scientific and was of Brockman Activity 1, 80-200 mesh. The methyl acetate used as solvent was supplied by Aldrich and was dried over molecular sieves. The yields were estimated by comparing the response of standard solutions to the response of solutions of the reaction products.

The results of these reactions are shown more graphically by the gas chromatograms. Figures 9 through 12 are gas chromatograms for the first fraction of reactions 1 through 4 of Table 2. For comparison, the gas chromatogram of the Army-furnished racemic soman standard is shown in Figure 8.

When the amine salt was reacted directly, the soman produced had an optical purity of only 12%. This is in line with the experience of other researchers when amine salts were used.(16) However, in the presence of alumina, the optical purity of the product increased to 36%. This is a significant increase and one of the first instances in which an amine salt has provided optically active material. In the absence of alumina, the sodium salt gave soman which we calculate to be only 23% optically pure. Again, in the presence of alumina there was a dramatic increase in optical purity to 58%.

### CONCLUSIONS

This program has demonstrated that at least one of the soman isomers can be prepared with a purity of approximately 75%. The method is

TABLE 2. REACTIONS OF THE SALTS OF C(+)<sub>2</sub>P(+)-O-PINACOLYL METHYLPHOSPHONOTHIOIC ACID WITH PICRYL FLUORIDE

Reaction	Salt	Amount of Salt, mole	Picryl Fluoride, mole	Alumina, grams	Fraction	$\frac{W[C(+)]P(-)]}{W[C(+)]P(+)]}$	W[C(+)]P(-)] %	W[C(+)]P(+)] %	Yield of Soman, %
1	Amine	0.0022	0.0044	None	1 <sup>a</sup>	1.30	56	44	--
2	Amine	0.0022	0.0044	0.5	1 2 <sup>b</sup>	2.09 1.94	68 66	32 34	--
3	Sodium	0.0022	0.0044	None	1 2	1.59 1.44	61 59	39 41	85
4	Sodium	0.0022	0.0044	0.5	1 2	3.67 3.14	79 76	21 24	100

<sup>a</sup> Collected while the reaction flask was kept at ambient temperatures.

<sup>b</sup> Collected while the reaction flask was heated to 45°-50°C.

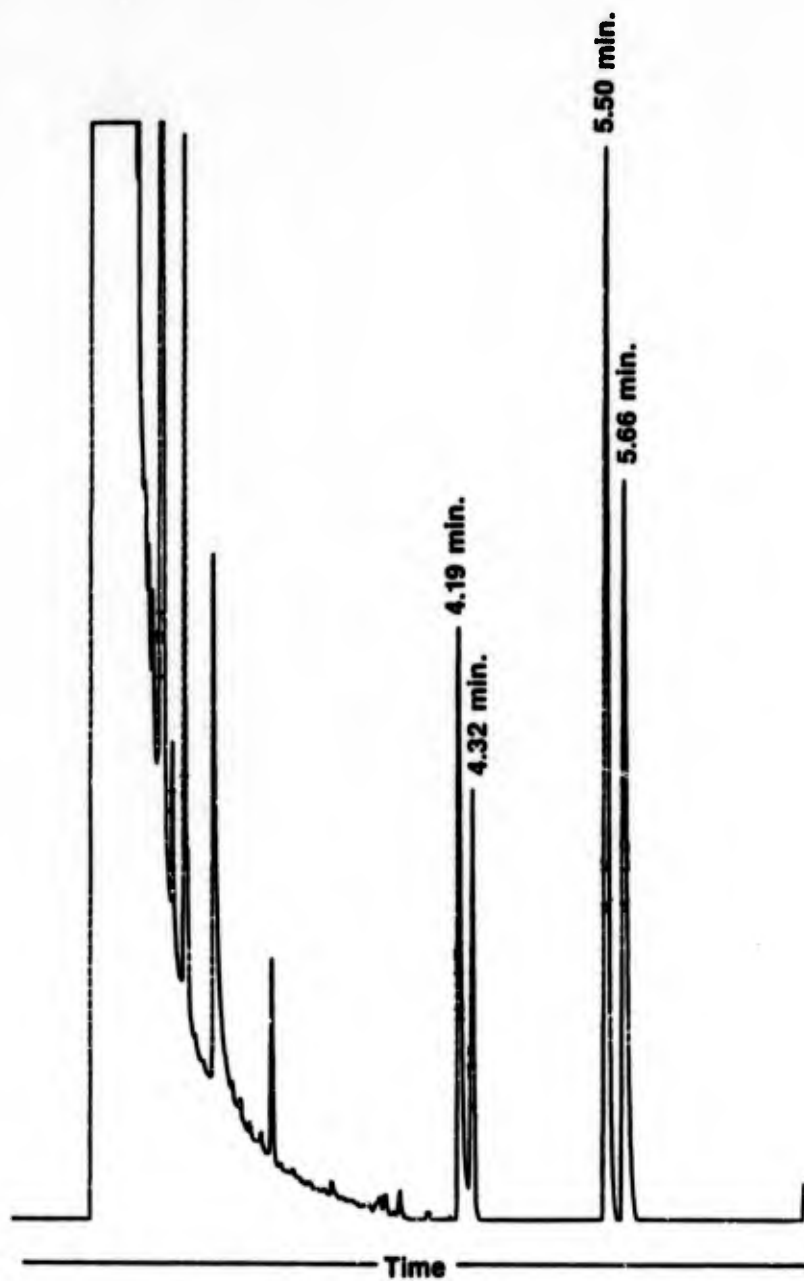


FIGURE 9. GAS CHROMATOGRAM OF SOMAN GENERATED IN REACTION 1

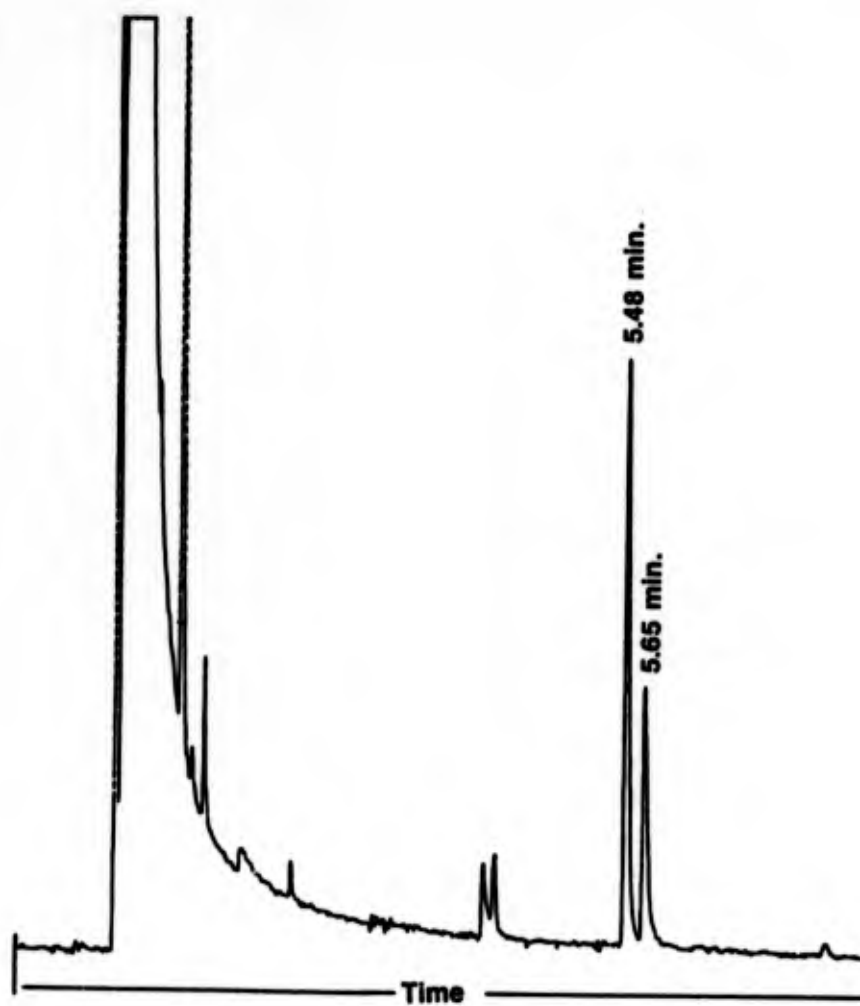


FIGURE 10. GAS CHROMATOGRAM OF SOMAN GENERATED IN REACTION 2

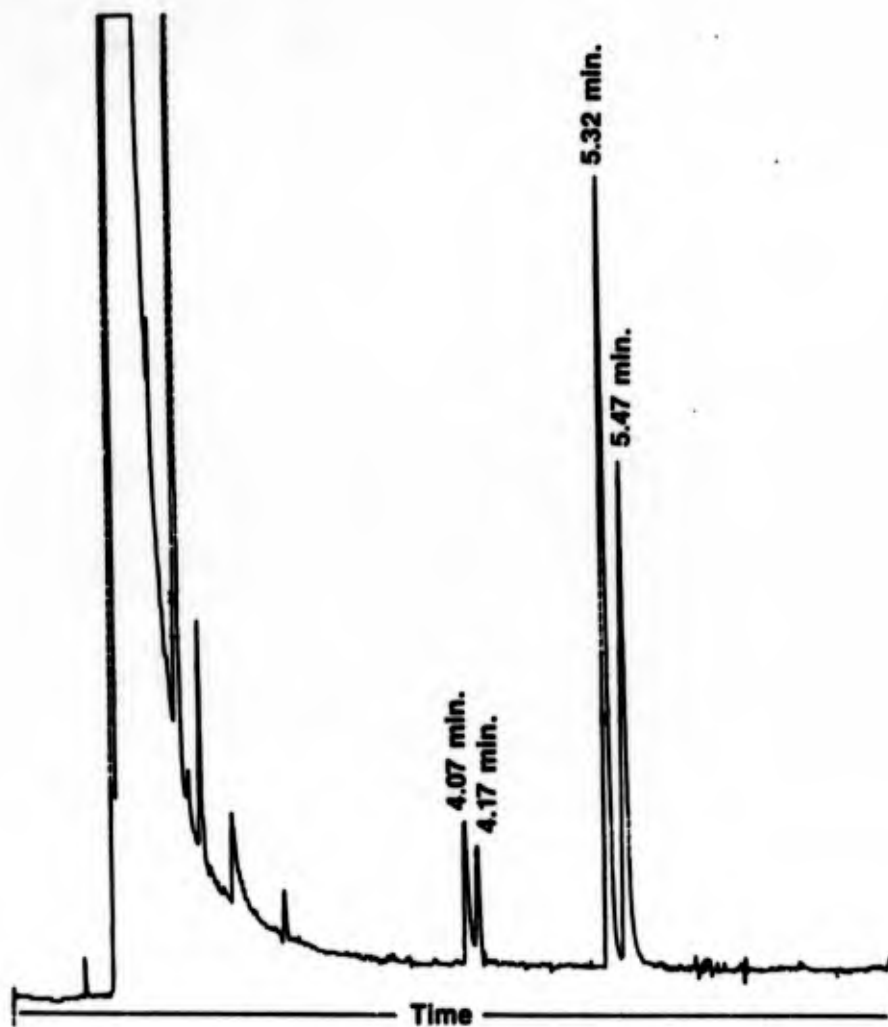


FIGURE 11. GAS CHROMATOGRAM OF SOMAN GENERATED IN REACTION 3

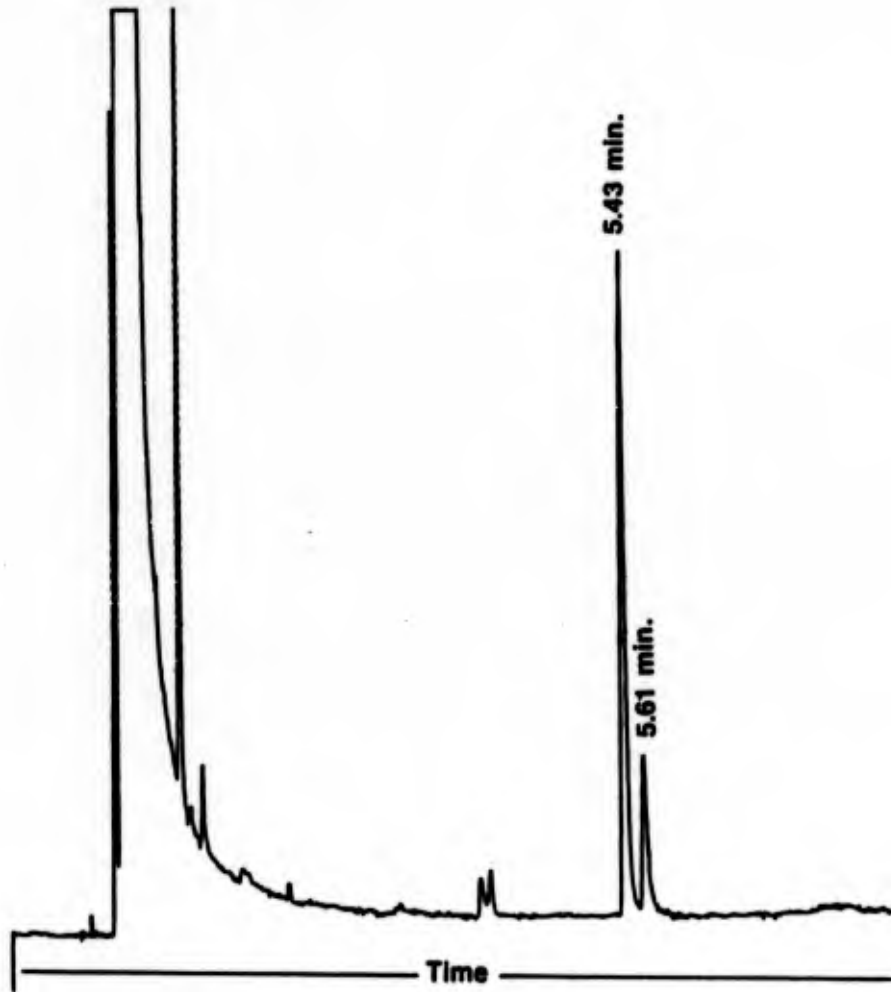


FIGURE 12. GAS CHROMATOGRAM OF SOMAN GENERATED IN REACTION 4

appropriate for all of the isomers and working on a scale that would produce gram quantities is entirely feasible.

Each of the four optical isomers of O-pinacolyl methylphosphonothioic acid has been prepared in quantity and is stored as the  $\alpha$ -phenethylamine salt. These compounds have been fully characterized and their anticholinesterase activity determined. With additional work, using ideas that have developed during this program, it may be possible to increase the optical purity of the soman isomers generated from these intermediates.

### RECOMMENDATIONS

The current program has shown that the goal of producing gram quantities of the optical isomers of soman by chemical means is feasible. To accomplish this goal, additional work is needed. Several promising leads developed during the course of this program are discussed below.

#### Fluoride Ion Trapping Agents

The presence of alumina in a reaction mixture that generates optical isomers of soman using picryl fluoride inhibits the racemization of the soman isomer produced. Only one type and amount of alumina was examined in this program. Several parameters need to be investigated, such as the amount of alumina, the kind of alumina (neutral, acidic) and the kind of absorbent (alumina, silica gel, boric acid). The use of such reagents offers real promise for generating optical isomers of high purity.

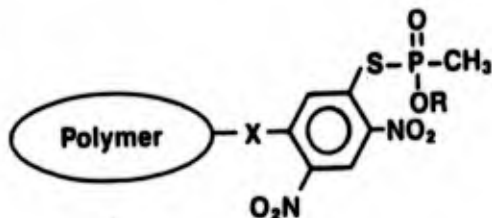
#### Counter Ion of Salt

The amine salts of optically active methylphosphonothioic acids, when reacted with picryl fluoride, lead to less pure optical isomers than do the corresponding sodium salts. We have speculated on the reasons for this and have concluded that using a smaller, harder counter ion such as lithium might also facilitate the preparation of isomers of higher purity.

#### Solid-Phase Derivative for Fluorination

In discussing the mechanism of the reaction of salts of optically active O-pinacolyl methylphosphonothioic acid with picryl fluoride, it was observed that the first stage of the reaction produced fluoride ion which is deleterious. If a polymer-bound reagent could be prepared by reacting the salt with a picryl fluoride analog, the fluoride

could then be removed prior to the second stage of the reaction. In addition, such a reagent would retain the by-products, giving essentially pure solutions of the soman isomer. A key intermediate is shown. The preparation of such a material is possible, as described in this report.



### Use of Chromatography with Synthesis

It has been demonstrated that the diastereomeric isomers of soman can be separated with baseline resolution using a particular capillary column. It is possible that a larger column could be developed that would separate the isomers in a similar way. Then, using a relatively pure optical isomer such as those prepared in this study, it would be possible to isolate highly purified isomers by preparative chromatography. It is possible that milligrams to hundreds of milligrams of purified isomer could be isolated in this way. This approach also needs to be investigated.

### EXPERIMENTAL PROCEDURES

Optical rotations were determined on a Bendix Corporation Series 1100 Automatic Polarimeter with a 1 cm cell. Melting points were determined on a Mel-Temp apparatus and are uncorrected. The NMR spectra were determined on a Varian CFT-20 80 Mhz spectrometer. The infrared spectra were determined on a Perkin-Elmer Model 421 grating spectrometer. Mass spectra were determined on a Finnigan system, Model 4500. Elemental analyses were performed by Galbraith Laboratories, Inc.

#### Resolution of Pinacolyl Alcohol. Preparation of (-)(R)-Pinacolyl Alcohol

A 2 liter, round-bottomed flask was charged with 50.0 g of D-alanine (Aldrich Chemical Co.), 147 g of racemic pinacolyl alcohol, 125 g of p-toluenesulfonic acid monohydrate, 785 ml of benzene and 340 ml of toluene. The mixture was refluxed under a Dean-Stark trap for 3 days.

Occasionally, a 10 g-aliquot of racemic pinacolyl alcohol was added during this period. The reaction was complete when a clear, homogeneous solution was produced. The volatiles were removed on a rotary evaporator and the resulting solid was slurried with 250 ml of ether, filtered and dried. The crude product was crystallized from 1 liter of benzene to give 84 g of material, melting at 174<sup>o</sup>-178<sup>o</sup>C. This material was crystallized twice more from benzene to give 60 g of the pure p-toluenesulfonate of the (-)-pinacolyl ester of D-alanine, mp 185<sup>o</sup>-186<sup>o</sup>C, [ $\alpha$ ]<sub>D</sub>-11.0<sup>o</sup> (C=5, methanol).

The ester salt was added to 150 ml of 5N sodium hydroxide solution and the mixture stirred for about 2 hours. Thin layer chromatography indicated the ester was completely reacted at this time. The reaction mixture was extracted three times with ether. The ether extracts were combined, washed with water and dried (MgSO<sub>4</sub>). The mixture was filtered and the ether distilled off through a 12 inch Vigreux column. The product was then distilled to give 16 g (95%) of (-)-pinacolyl alcohol, bp 108<sup>o</sup>-112<sup>o</sup>C, [ $\alpha$ ]<sub>D</sub>-4.8<sup>o</sup> (C=5, ethanol).

#### C(+)-O-Pinacolyl Methylphosphonothioic Acid

The preparation described here starts with (+)-pinacolyl alcohol. The C(-)-compound was prepared in the same way, starting with (-)-pinacolyl alcohol.

(+)-Pinacolyl alcohol, 20.0 g (0.196 mole), was dissolved in 100 ml of benzene in a 1 liter, 3-necked round-bottomed flask equipped with a thermometer, a 125 ml dropping funnel, drying tube and stirring bar. To this solution was added 17.9 ml, 23.4 g (0.20 mole), of methylchlorophosphine in 100 ml of benzene dropwise over 30 minutes. Occasional cooling with an ice bath was necessary in order to keep the temperature below 35<sup>o</sup>C. Most of the addition was carried out at a temperature between 30<sup>o</sup> and 35<sup>o</sup>C. The mixture was stirred for 3 hours.

Sodium bicarbonate, 33 g, was added in portions over 1 hour. There was a steady mild exotherm and noticeable CO<sub>2</sub> evolution. An additional 33 g of sodium bicarbonate was added over the next hour. However, during that period CO<sub>2</sub> evolution became almost violent and the reaction mixture was occasionally cooled to keep it under control. After 2 hours, the reaction subsided and no additional CO<sub>2</sub> evolution was observed. The mixture was stirred an additional 1/2 hour and then allowed to sit overnight.

The mixture was filtered and the salts were washed with a small amount of benzene. The filtrate and washes were transferred to a 500 ml, 3-necked round-bottomed flask equipped with a thermometer, a gas inlet, a drying tube and a stirring bar. To this solution was added 6.3 g (0.20 mole) of sublimed sulfur and anhydrous ammonia was bubbled in (below the surface of the mixture) with stirring. In 40 minutes all the sulfur had dissolved and the temperature had reached 35<sup>o</sup>C. As ammonia addition

continued, a precipitate began to form and eventually the reaction mixture became so thick that stirring was difficult. After 2 hours, addition of ammonia was stopped. The reaction mixture was transferred to a 500 ml separatory funnel and extracted 3 times with a total of 400 ml of water. The water extracts were combined, washed once with benzene and then acidified with concentrated hydrochloric acid to pH 1. The free acid was extracted into ether (3 extractions), a total of 350 ml. The ether extracts were combined, washed twice with water and dried ( $\text{MgSO}_4$ ). After filtration the ether was removed on the rotary evaporator. The colorless liquid weighed 25.6 g (67% yield). The C(+)-O-pinacolyl methylphosphonothioic acid appeared by NMR to be pure and was used in the next procedure without further purification.

Resolution of C(+)-O-Pinacolyl Methylphosphonothioic Acid  
Preparation of the (+)- $\alpha$ -Phenethylamine Salt of C(+)-P(+)-O-  
Pinacolyl Methylphosphonothioic Acid

The C(+)-acid prepared as above, 24 g (0.12 mole), was dissolved in 100 ml of anhydrous ether and then with ice bath cooling, a solution of 7.3 g (0.060 mole) of (+)- $\alpha$ -phenethylamine (Alfa Chemical Co.) in 100 ml of ether was added dropwise over about 20 minutes. The reaction mixture was allowed to sit overnight and the white solid filtered off and washed with a small amount of ether. The filtrate and washes were combined for preparation of the C(+)-P(-)-isomer described later.

The white solid, crude C(+)-P(+), A(+), weighed 14.4 g (75%), mp  $187^\circ\text{--}189^\circ\text{C}$ ,  $[\alpha]_D^{25} +14.0^\circ$  (C=6, methanol). Crystallization of 14 g of this product from 700 ml of ethyl acetate provided 12.7 g (68% yield) of pure C(+)-P(+), A(+), mp  $190^\circ\text{--}191^\circ\text{C}$ ,  $[\alpha]_D^{25} +14.6^\circ$  (C=6, methanol). Additional crystallizations did not increase the melting point or the optical rotation. This material gave a satisfactory elemental analysis (see text).

Preparation of the (-)- $\alpha$ -Phenethylamine Salt of  
C(+)-P(-)-O-Pinacolyl Methylphosphonothioic Acid

The filtrate described above, containing primarily C(+)-P(-)-acid, was treated as follows: The volatile compounds were removed on the rotary evaporator and the remaining yellow oil, which weighed 15 g, was cooled in an ice bath and treated with 75 ml of 2 N sodium hydroxide solution. The resulting mixture was extracted 3 times with benzene to remove any (+)- $\alpha$ -phenethylamine. The aqueous solution was acidified with concentrated hydrochloric acid to pH 1. The resulting mixture was extracted 3 times with a total of 75 ml of ether to remove the free acid. The ether extracts were combined, washed twice with water and dried ( $\text{MgSO}_4$ ). The mixture was filtered and the ether removed on the rotary evaporator, leaving 11 g of crude C(+)-P(-) acid.

The crude acid was dissolved in 100 ml of ether and treated dropwise with a solution of 7.3 g of (-)- $\alpha$ -phenethylamine in 100 ml of ether with ice-cooling over 25 minutes. After addition, the reaction mixture was allowed to sit overnight. The white solid was filtered off, washed with a small amount of ether and dried. The crude product weighed 12.3 g (64%), mp 171<sup>o</sup>-174<sup>o</sup>C,  $[\alpha]_D$ -2.8<sup>o</sup> (C=6, methanol). This material, 12.0 g, was crystallized from about 180 ml of ethyl acetate. The pure C(+) $P(-)$ , A(-), compound weighed 10.8 g (58% yield), mp 173.5<sup>o</sup>-174.5<sup>o</sup>C,  $[\alpha]_D$ -2.9<sup>o</sup> (C=6, methanol). Additional crystallizations did not increase the melting point or the optical rotation. A satisfactory elemental analysis is reported in the text of this report.

The same procedure was used to prepare the other optical isomers, C(-) $P(-)$ , A(-), and C(-) $P(+)$ , A(+), starting with C(-)-O-pinacolyl methylphosphonothioic acid, so these will not be described separately.

#### Preparation of the Sodium Salt of C(+) $P(+)$ - O-Pinacolyl Methylphosphonothioic Acid

The (+)- $\alpha$ -phenethylamine salt of C(+) $P(+)$ -O-pinacolyl methylphosphonothioic acid described above, 2.0 g (6.3 mmole), was suspended in 60 ml of distilled water and the stirred solution adjusted to pH 1 with concentrated hydrochloric acid. The mixture was extracted 3 times with ether. The ether extracts were combined, washed twice with water, dried ( $MgSO_4$ ) and filtered. Removal of the solvent on the rotary evaporator left 1.17 g (94%) of colorless liquid.

The C(+) $P(+)$  acid prepared as described above, 1.7 g (8.66 mmole) was dissolved in 20 ml of tetrahydrofuran (THF) and this solution added dropwise under nitrogen to 260 mg (10.8 mmole) of sodium hydride covered with 10 ml of THF over 30 minutes. The mixture was stirred for 15 minutes and then filtered. The solvent was removed from the filtrate and the residue treated with benzene. This treatment consisted of dissolving the residue in benzene and then evaporating the solvent. This treatment was repeated until the residue became a solid. The solid was then dried to a constant weight under vacuum. The yield was quantitative. Different batches had different melting points. The highest melting point obtained was 237<sup>o</sup>-238<sup>o</sup>C; the lowest was 231<sup>o</sup>-233<sup>o</sup>C.

#### Attempts to Prepare C(+) $P(-)$ -Soman

This reaction was carried out in Battelle's Hazardous Materials Laboratory. Because of the extreme toxicity of soman and its isomers, any attempt to produce soman should be carried out only by specially trained personnel in a laboratory designed for chemical surety materials and with trained medical personnel available.

This is a description of reaction 4, described in the text. The other reactions were carried out in a similar manner. The sodium salt of C(+)-P(+)-O-pinacoyl methylphosphonothioic acid, 0.48 g (2.2 mmole), was placed in a 25 ml round-bottomed flask containing a stirring bar and equipped with a reflux condenser with a drying tube. To this flask was then added 0.5 g of alumina and 1.02 g (4.4 mmole) of picryl fluoride. All at once 3.0 ml of anhydrous methyl acetate was added and stirring initiated after the condenser was replaced. The mixture immediately turned deep red. Stirring was continued for 30 minutes. The reflux condenser was replaced with a distillation head attached to a 10 ml receiver. A water bath at ambient temperature was placed under the reaction flask and a dry ice/acetone bath was placed under the receiver. The solvent and as much soman as possible were distilled by gradually decreasing the pressure to 0.25 mm Hg and maintaining it there for 10 minutes. The vacuum was released and the receiver (fraction 1) replaced with another 10 ml round-bottomed flask containing 3.0 ml of methyl acetate. The dry ice/acetone bath was placed under the receiver. Distillation was resumed at 0.20 mm Hg as the water bath under the reaction flask was heated to 50°C over 10 minutes. It was maintained at 50°C for 20 minutes. It was during this period that most of the soman distilled over. The second receiver was then removed and the solution analyzed as fraction 2.

Each of the fractions was then prepared for analysis by GLC by removing a 10 µl aliquot of the distillate and diluting it to 3.0 ml. The second fraction had to be diluted another 10-fold in order to bring the concentration to a level comparable to that of the standard which was 25 µg/ml. The results of these analyses and a discussion are contained in the text of this report.

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