GAS CHROMATOGRAPHIC ANALYSIS OF THE STEREOISOMERS OF THE CHEMICAL WARFARE AGENT GF

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ABSTRACT

Synthesis of the nerve agent cyclohexyl methylphosphonofluoridate (GF) yields a racemic mixture of two stereoisomers due to the presence of an asymmetric phosphorus atom. These two enantiomers, designated as P(+) and P(-), were separated in 8 minutes by chiral gas chromatography and quantified using flame photometric detection. Plots of peak area versus concentration of the individual enantiomers resulted in correlation coefficients greater than 0.99. The enantiomers were present in racemic GF in the ratio of 3:2 as P(-):P(+). The P(-) enantiomer was isolated using a method whereby the P(+) isomer was removed from the racemic mixture via preferential enzymatic catalyzed hydrolysis. The specific rotation of the P(-) enantiomer at 589 nm and 25 °C in methylene chloride was calculated to be −19.3°. The P(-) enantiomer also inhibited acetylcholinesterase more strongly than the racemic mixture, consistent with the findings for other optically active nerve agents.

INTRODUCTION

The chemical warfare agents GA, GB, GD, GF, and VX all have asymmetric phosphorus atoms resulting in pairs of P(+) and P(-) stereoisomers. GD also has an asymmetric carbon, which gives it four stereoisomers. Previously reported data on GA, GB, GD, and VX show that most of the toxicity of these compounds, as measured by inhibition rate of acetylcholinesterase (AchE), is due to the P(-) isomers.1-5 No data have yet been published on the toxicities of the individual GF enantiomers. The enantiomers for all four of the G-agents have been separated by chiral gas chromatography (GC).6 VX enantiomers were resolved using a chiral liquid chromatography method.7 Separation of the GF enantiomers proved to be the most challenging, with retention times in excess of 80 minutes.8 A chiral GC method was developed to perform rapid quantitative measurements of the enantiomers of GF. The work described here was part of an investigation into the stereospecificity of nerve agent degrading enzymes.9,10
Gas Chromatographic Analysis Of The Stereoisomers Of The Chemical Warfare Agent Gf

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EXPERIMENTAL

The gas chromatograph was a Hewlett-Packard model 6890 equipped with a flame photometric detector and an automatic sampler. The conditions were as follows:

Column: 25m X 250 um id X 0.12 um Chrompack Chirasil-Val-L.
Injection volume: 1 microliter, 100:1 split.
Inlet temperature: 200 °C.
Oven temperature: 90 °C (isothermal).
FPD temperature: 200 °C (phosphorus mode).
Carrier (helium) flow rate: 1 ml/min.

A chromatographically pure preparation of a single isomer of GF was made by degrading the isomer on which the enzyme had the greater activity. The enzymatic reaction was run at 15 °C and pH 7.0 in order to minimize spontaneous hydrolysis. Just past the midpoint deflection, the reaction was extracted with methylene chloride and the extract was analyzed by GC using the above conditions. A single peak was observed representing a single GF isomer. When NaF was shaken with a solution of this isomer, subsequent GC analysis showed two peaks, consistent with NaF catalyzed racemization of GF. Since the racemization occurs in the absence of enzyme, the direct effect of NaF on GF appears to be at least one means by which GF is racemized.

The specific rotation of the single GF isomer was then calculated based on measurements of the observed rotation made at 589 nm (sodium line) and 25 °C using a Perkin Elmer 141 Electronic Polarimeter and a sample cell with a path length of 10 cm.

RESULTS AND DISCUSSION

The enantiomers of GF were consistently separated by 0.15 minutes at retention times near 8 minutes. A typical chromatogram is shown in figure 1a. Hydrolysis of the racemic mixture yielded the single isomer shown in figure 1b. Optical rotation measurements later revealed this to be the P(−) isomer, the specific rotation at 589 nm and 25 °C being calculated as -19.3°. The isomers were consistently present in the ratio P(−):P(+) of 3:2 based on peak area.

GA, GB, GD, and VX are known to stereospecifically bind acetylcholinesterase (AChE) resulting in a significant difference in toxicity between enantiomers.1–5 Nerve agents and enzymes all exert their effects in a biological, chiral environment, so it should be expected that those effects would be stereoselective. For these agents, the P(−) isomers are all more toxic than the P(+) isomers. In the case of GA, the (−) isomer is 7 times more toxic than the (+) isomer.1,2 Racemic GB is half as toxic as (−) GB, indicating that essentially all the toxicity is derived from the P(−) isomer.1,2 Essentially all the toxicity of GD is derived from the two P(−) isomers.2 The (−) isomer of VX is 13 times more toxic than the (+) isomer.4 Figure 2 shows the results of an AChE inhibition assay in the presence of racemic and (−) GF as compared to spontaneous inhibition. The decrease in product yield is a result of the degree of binding of GF to AChE, or the increased inhibition of the enzyme. The rate of product formed is approximately halved in the
Figure 1. Gas Chromatograms of Racemic GF and (-)GF.

Figure 2. AChE Inhibition by GF.
presence of (-)GF when compared to racemic GF. This shows that pure (-)GF is more toxic than the racemic mixture.

Although baseline separation was not achieved, the separation between (-) and (+) GF was sufficient to quantitatively measure these isomers. Attempts to achieve baseline resolution of the enantiomers by modifying the GC conditions proved unsuccessful. Figure 3 shows sample plots of peak area versus concentration of the stereoisomers, as calculated from the ratio of peak area of the individual enantiomers to that of the racemic agent. Linear regression analyses of these data yielded correlation coefficients greater than 0.99.

**CONCLUSIONS**

A rapid and quantitative chiral GC method was developed to measure the individual stereoisomers of GF with retention times near 8 minutes. The P(-) enantiomer was successfully purified from the racemic agent and its specific rotation at 589 nm and 25 °C in methylene chloride was calculated to be −19.3°. The P(-) isomer of GF was shown to be more toxic than the racemic mixture and, consequently, much more toxic than the P(+) isomer. This is consistent with the findings for other nerve agents with optically active isomers.
REFERENCES


