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Stereochemical Analysis of the Nerve Agents Soman, Sarin, Tabun, and VX by Proton NMR-Spectroscopy with Optically Active Shift Reagents

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Those anticholinesterases of the organophosphate type which are considered to be potential chemical warfare agents (Franke 1977) have a center of asymmetry at the phosphorus atom. Consequently, the agents are mixtures of two enantiomeric forms designated as P(+)- and P(-)-isomers (P = phosphorus). Examples of such nerve agents are 1,2,2-trimethylpropyl methylphosphonofluoridate (1,X=0;soman), isopropyl methylphosphonofluoridate (2; sarin), ethyl dimethylphosphoramidocyanidate (3; tabun), and ethyl S-diisopropylaminoethyl methylphosphonothioate (4; VX). Soman (1, X=0) has an additional chiral center in the 1,2,2-trimethylpropyl (pinacolyl) moiety. Hence, soman consists of four stereoisomers designated as C(-)P(-), C(-)P(+), C(+)P(+), and C(+)P(-) (C = carbon) (Benschop et al. 1981a).

For sarin, soman and agents related to VX, it has been shown that their stereoisomers may vary widely in their rates of inhibition of acetylcholinesterase, overall toxicity, and in various other relevant toxicological properties (Benschop et al. 1981b, 1984; Boter et al. 1971). We attempt here to develop analytical procedures which allow identification and quantification of the separate stereoisomers of nerve agents. Recently, we reported a procedure to analyze the four stereoisomers of soman (1,X=0) based on capillary GLC with a Carbowax column coupled to a column coated with optically active Chirasil Val. (Benschop et al. 1981a). However, this system does not separate the enantiomers of sarin (2), tabun (3), and VX (4). Since all agents 1-4 have a phosphoryl bond which associates strongly with lanthanide NMR shift reagents (Ward et al. 1971), it occurred to us that the ^{1}H-NMR spectra of 1-4 in the presence of optically active shift reagents might allow separate observation of the stereoisomers (Sullivan 1978). In this paper we report our positive results using the shift reagents tris(3-trifluoroacetyl-d-camphorato)

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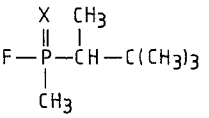
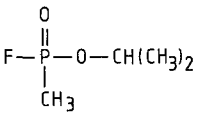


1, soman (X=O)



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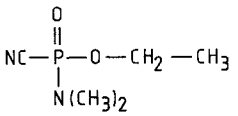
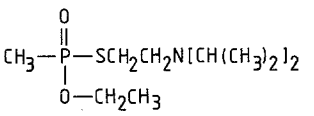


3, tabun



4, VX

europium (III), [Eu(tfc)$_3$], and tris (3-heptafluorobutyryl-d-camphorato) europium (III), [Eu (hfc)$_3$].

MATERIALS AND METHODS

The achiral NMR shift reagent tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium (III), [Eu(fod)$_3$], and the optically active shift reagents tris (3-trifluoroacetyl-d-camphorato) europium (III), [Eu(tfc)$_3$], and tris (3-heptafluorobutyryl-d-camphorato) europium (III), [Eu(hfc)$_3$], were obtained from EGA/Aldrich (Weinheim, W.-Germany). These reagents were used without further purification and were handled under anhydrous conditions.

The nerve agents soman (1, X=O), sarin (2), tabun (3), and VX (4) were prepared according to standard procedures (Franke 1977) in our laboratory. C(-)P(±)-soman and C(±)P(+)-soman were obtained by means of previously developed procedures (Benschop et al. 1984). The work-up procedure for C(±)P(+)-soman was adapted for the use of solvents, suitable for ^{1}H-NMR analysis, i.e. the C(±)P(+)-isomers remaining in aqueous phosphate buffer after stereospecific inhibition of chymotrypsin were extracted directly with carbon tetrachloride. 1,2,2-Trimethylpropyl methylphosphonofluoridothionate (1, X=S) was prepared by the procedure of Boter and Ooms (1966) and was purified by spinning band distillation to 99% purity (glc). Dextrorotatory sarin (30% ee) in methyl acetate solution was prepared according to the procedure of Boter et al. (1966). For ^{1}H-NMR-analysis, the solvent was replaced by carbon tetrachloride by means of repeated addition of carbon tetrachloride and concentration in vacuo. (R)-(+)-VX (neat) was prepared according to the procedure of Boter and Platenburg (1967).

In the standard procedure for measurement of the ^{1}H-NMR spectra, the appropriate volume of shift reagent dissolved in carbon tetrachloride (ca 70 mM) was added from a glass-tipped† "Capiletor" (Clinicon International GMBH, W.-Germany) to 0.5 ml of a solution of the nerve agent (ca 15 mM) in carbon tetrachloride/deuteriochloroform (3/1, v/v) contained in a 5 mm cylindrical NMR sample tube (Wilmad 507 PP). Deuteriochloroform was added as an internal lock for the measurement of the ^{1}H-NMR spectra. Tetramethylsilane was used as internal standard. Usually, optimal spectra were obtained when the molar ratio nerve agent/shift reagent was in the range 1.8-2.5. ^{1}H-NMR spectra were recorded at a frequency of 100.1 MHz in the Fourier transform mode on a Varian XL 100-12 NMR spectrometer system. A spectral width of 1024 Hz, a 90° pulse (pulse width 20 µs) and an acquisition time of 4 s were applied.

RESULTS AND DISCUSSION

^{1}H-NMR spectra of the nerve agents 1-4 were recorded in the absence and in the presence, respectively, of the optically active europium shift reagents Eu(tfc)$_3$ or Eu(hfc)$_3$. In order to facilitate interpretation, spectra were also recorded in the presence of the achiral shift reagent Eu(fod)$_3$. In all cases the expected downfield shift ($\Delta\delta$) of the organophosphate hydrogen atoms, due to association of the europium shift reagent with the oxygen atom of the phosphoryl group was observed. This shift increased with an increasing molar ratio shift reagent/nerve agent, and decreased with increasing distance of the observed hydrogen atom from phosphoryl oxygen. When the phosphoryl group of soman (1, X=O) is replaced by thiophosphoryl (1, X=S) a shift reagent induced downfield shift is not observed. Obviously, the oxygen atom of the phosphoryl group in (1, X=O) is the only site available for association with shift reagents (cf. Ward et al. 1971). The magnitude of the shift differences observed between enantiomers in the presence of chiral shift reagent ($\Delta\Delta\delta$) and the observed coupling constants are summarized in the Table.

The ^{1}H-NMR spectrum of soman (1, X=O) (Figure 1A) shows the quartet of the P-CH_3 hydrogens due to coupling with phosphorus (J=18.7 Hz) and coupling with fluorine (J=5.6 Hz), in addition to the doublet of the CH-C$\underline{H}_3$ (J=6.4 Hz) and the singlet of the C$(CH_3)_3$ hydrogens. Upon addition of Eu(fod)$_3$ (molar ratio soman/shift reagent=1.9), all hydrogen peaks shift downfield (Figure 1B). Due to peak broadening the P-CH_3 peaks are no longer resolved with regard to the H-F coupling. The two diastereoisomeric pairs of soman become visible in the doubling of the C$(CH_3)_3$ singlet and of the CH-C$\underline{H}_3$ doublet. Replacement of

†When plastic tips are used, precipitates are formed in the solution.

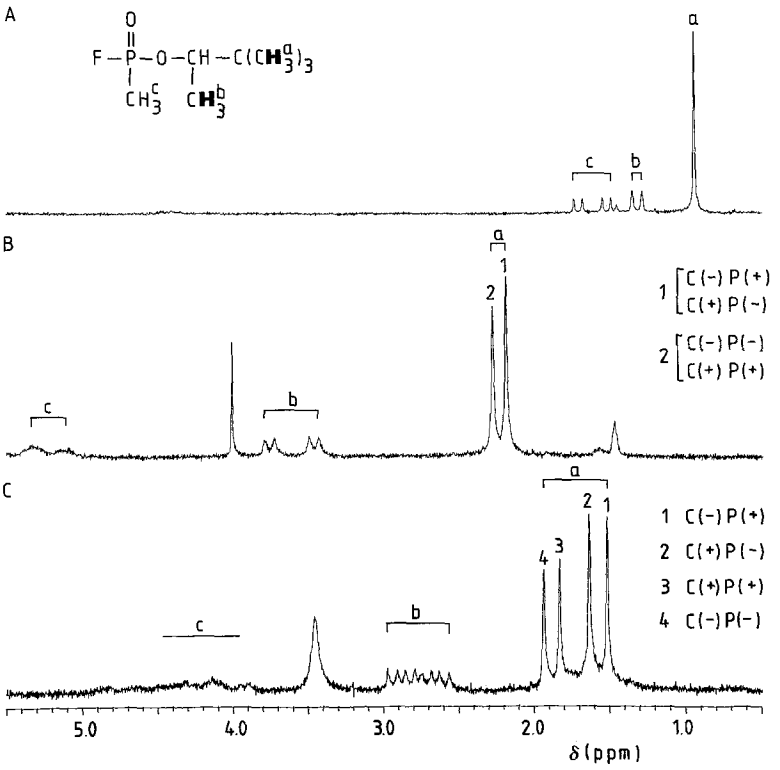


Figure 1. ^{1}H-NMR spectra (100 MHz) of 13.6 mM 1,2,2-trimethylpropyl methylphosphonofluoridate (1,X=O; soman) in $CCl_4/CDCl_3$ (3/1, v/v). A: no shift reagent added; B: achiral $Eu(fod)_3$ added (7.0 mM); C: optically active $Eu(tfc)_3$ added (6.3 mM).

$Eu(fod)_3$ by optically active $Eu(tfc)_3$ in approximately the same molar ratio to soman leads to a further splitting of the CH-$C\underline{H}_3$ hydrogens to eight peaks and of the $C(CH_3)_3$ hydrogens to four peaks (Figure 1C). Obviously, the four stereoisomers of soman become apparent in this spectrum. It is evident that the relatively intense and sharp signals of the $C(CH_3)_3$ hydrogens, each corresponding with one stereoisomer, are most suitable for analytical purposes. These peaks were partly identified in the ^{1}H-NMR spectrum of C(-)P(±)-soman in the presence of $Eu(tfc)_3$. This mixture of C(-)P(+)- and C(-)P(-)-soman has only the two outer peaks of the four pertaining to the hydrogens of the $C(CH_3)_3$ group, as was shown by subsequent addition of C(±)P(±)-soman. A further identification of the $C(CH_3)_3$ peaks was obtained from the ^{1}H-NMR spectrum of a mixture of the $Eu(tfc)_3$-associated P(+)-isomers of soman, obtained by stereospecific inhibition of chymotrypsin with the P(-)-isomers of C(±)P(±)-soman (Benschop et al.

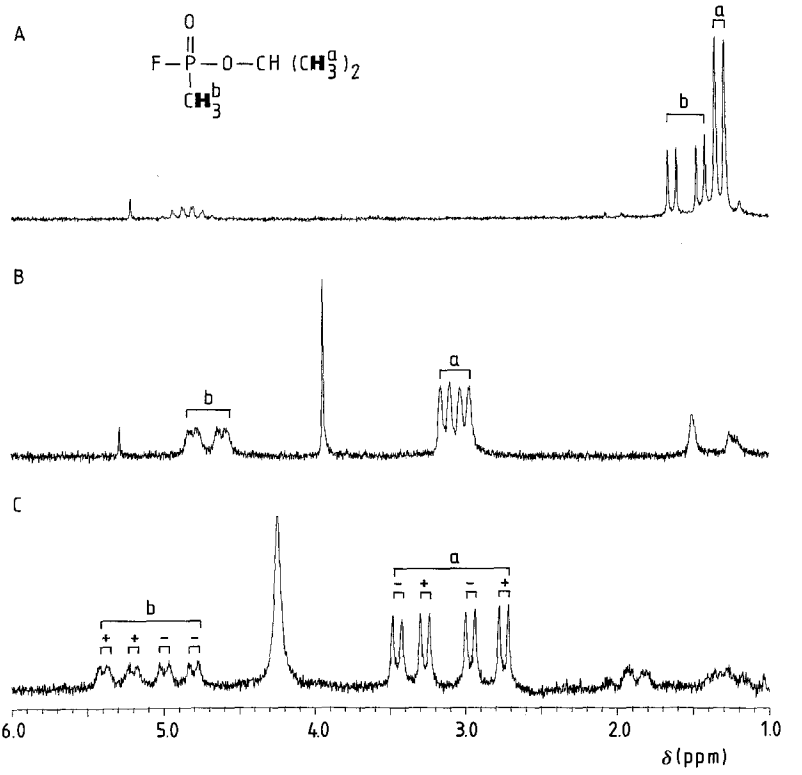


Figure 2. ^{1}H-NMR spectra (100 MHz) of 14.5 mM isopropyl methylphosphonofluoridate (2; sarin) in $CCl_4/CDCl_3$ (3/1, v/v). A: no shift reagent added; B: achiral $Eu(fod)_3$ added (7.0 mM); C: optically active $Eu(hfc)_3$ added (6.9 mM).

1984). This mixture of C(-)P(+)- and C(+)P(+)-soman, obtained in a ratio of 1.6:1 as shown by gas chromatography on the Carbowax-Chirasil Val system, appeared in approximately the same ratio as peaks 1 and 3, respectively, in the ^{1}H-NMR spectrum of the $Eu(tfc)_3$-associated sample (cf. Figure 1C), and as peaks 1 and 2, respectively, in the spectrum of the $Eu(fod)_3$-associated sample (cf. Figure 1B). Hence, the two pairs of enantiomers of soman are identified in the four peaks of the $C(CH_3)_3$ group of $Eu(tfc)_3$-associated soman as shown in Figure 1C, as well as in the two peaks of the $C(CH_3)_3$ group of $Eu(fod)_3$-associated soman (Figure 1B). In this way, the stereoisomers of soman are readily identified and semi-quantitatively analyzed, provided that the concentration of the stereoisomers is $\geq$ 1 mM. At this concentration limit, approximately 2.10^3 accumulations, taking 2.2 h, are needed to obtain an interpretable spectrum. Racemization of soman-

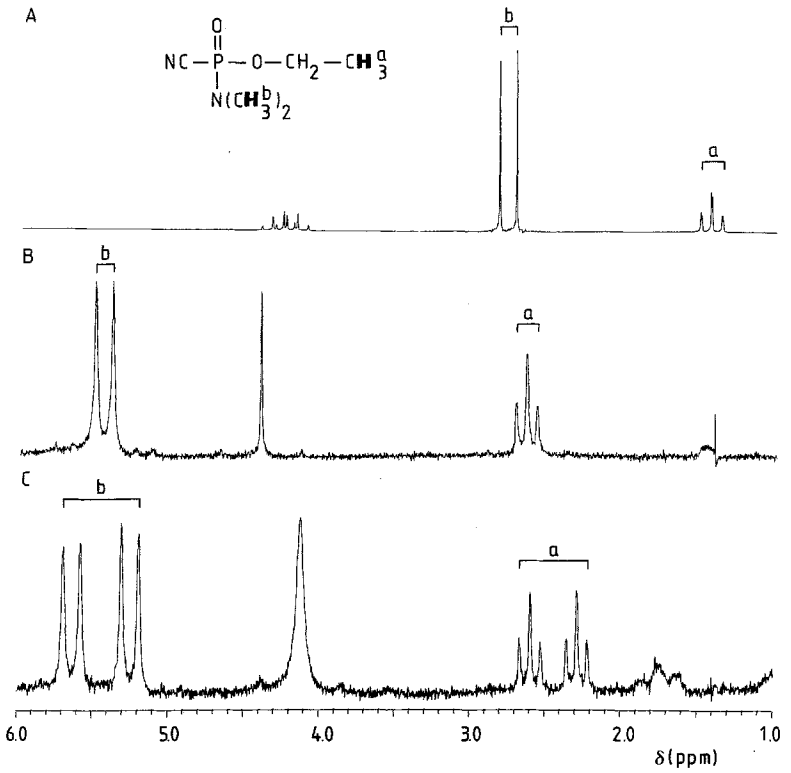


Figure 3. ^{1}H-NMR spectra (100 MHz) of 13.5 mM ethyl dimethylphosphoramidocyanidate (3; tabun) in $CCl_4/CDCl_3$ (3/1, v/v). A: no shift reagent added; B: achiral $Eu(fod)_3$ added (7.0 mM); C: optically active $Eu(hfc)_3$ added (6.9 mM).

stereoisomers in the presence of $Eu(tfc)_3$ was not observed when the solutions were kept overnight at room temperature, although some decomposition of soman is observed under these conditions.

As in soman, the NMR signals of the P-CH_3 hydrogens of sarin (2) in $CCl_4/CDCl_3$ split up into a quartet due to coupling with phosphorus and with fluorine (Figure 2A). The diastereotopic (Mislow and Raban 1967) methyl groups in the isopropyl moiety of sarin cannot be distinguished in this spectrum (J_{HH}=6.1 Hz). However, upon addition of the achiral shift reagent $Eu(fod)_3$ in a molar ratio sarin/shift reagent = 2.1, the downfield shifting doublet of these methyl groups splits up into two doublets as shown in

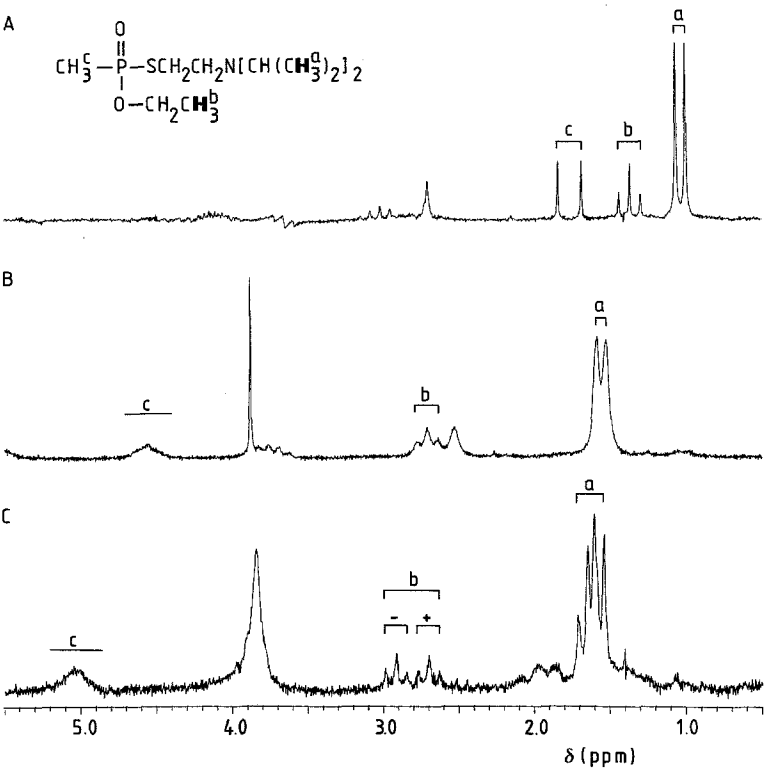


Figure 4. ^{1}H-NMR spectra (100 MHz) of 12.9 mM ethyl S-diisopropylaminoethyl methylphosphonothioate (4; VX) in $CCl_4/CDCl_3$ (3/1, v/v). A: no shift reagent added; B: achiral $Eu(fod)_3$ added (10.6 mM); C: optically active $Eu(hfc)_3$ added (10.5 mM).

Figure 2B, demonstrating their diastereotopic nature. Replacement of $Eu(fod)_3$ by optically active $Eu(hfc)_3$ in the same molar ratio splits up each of these doublets into a quartet, resulting in eight clearly separated peaks (Figure 2C). A doubling of peaks, although less explicitly observed due to peak broadening, is also obtained for the four peaks of the P-CH_3 hydrogens. Evidently, association of sarin with $Eu(hfc)_3$ allows separate observation of the two enantiomers of this nerve agent in the ^{1}H-NMR spectrum. This conclusion is experimentally confirmed by the spectrum of $Eu(hfc)_3$-associated dextrorotatory sarin (30% ee), which readily permitted stereoisomeric identification of the peaks as shown in Figure 2C.

In the ^{1}H-NMR spectrum of tabun (3) in $CCl_4/CDCl_3$ (Figure 3A) the triplet of the C-CH_3 hydrogens and the doublet of the $N(CH_3)_2$

TABLE. Coupling constants (J) and separation of the ^{1}H-NMR signals of the enantiomers ($\Delta\Delta\delta$) of the nerve agents soman (1, X=O), sarin (2), tabun (3), and VX (4), dissolved in $CCl_4/CDCl_3$ (3/1, v/v), in the presence of the optically active shift reagents $Eu(tfc)_3$ or $Eu(hfc)_3$.

Nerve agent	Observed hydrogen	Coupling constants[a] (Hz)	Shift reagent	Molar ratio nerve agent/ shift reagent	$\Delta\Delta\delta$
C(-)P(-) soman C(+)P(+)	$C(CH_3)_3$		$Eu(tfc)_3$	2.2	0.10
C(+)P(-) soman C(-)P(+)	$C(CH_3)_3$		$Eu(tfc)_3$	2.2	0.12
sarin	$C(CH_3)_2$	J_{HH}= 6.0(6.1)	$Eu(hfc)_3$	2.1	0.18–0.22[b]
	P-CH_3	J_{HP}=19.3(18.8) J_{HF}= 5.4(5.6)			0.40
tabun	$N(CH_3)_2$	J_{HP}=11.4(11.4)	$Eu(hfc)_3$	2.0	0.39
	C-CH_3	J_{HH}= 7.0(7.1)			0.31
VX	$CH_2\underline{C}H_3$	J_{HH}= 7.0(7.1)	$Eu(hfc)_3$	1.2	0.15

[a]Values between brackets refer to coupling constants in the absence of shift reagent. [b] Inequivalence due to diastereotopic methyl groups.

hydrogens shift downfield upon addition of half the equimolar amount of $Eu(fod)_3$ (Figure 3B). Replacement of $Eu(fod)_3$ by optically active $Eu(hfc)_3$ in the same molar ratio to tabun leads to a doubling of these peaks (Figure 3C), obviously due to the two enantiomeric forms of tabun†. To our knowledge, the ^{1}H-NMR spectrum as shown in Figure 3C is the first experimental observation of chirality in tabun. A further identification of the peaks could not be obtained, since reproducible methods to prepare optically active tabun are not yet available (Augustinsson 1957).

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†Qualitatively the same split-up is observed upon addition of a 10-fold molar excess of optically active methylphenylphosphinothioic acid to a 15 mM solution of tabun in $CCl_4/CDCl_3$ (cf. Harger 1980).

The "optical resolution" in the ^{1}H-NMR spectrum of the nerve agent VX (4) upon association with the optically active shift reagents Eu(hfc)$_3$ or Eu(tfc)$_3$ is unsatisfactory due to pronounced broadening and incomplete resolution of the peaks. The least unsatifactory result is obtained with Eu(hfc)$_3$-associated VX. The triplet of the CH_2-$C\underline{H}_3$ hydrogens shifts downfield by addition of achiral Eu(fod)$_3$ in a molar ratio VX/shift reagent = 1.2 (Figure 4B), and is doubled by replacement of this shift reagent by optically active Eu(hfc)$_3$ in the same molar ratio (Figure 4C). In a merely qualitative way, this allows separate recognition of the two enantiomers of VX. The stereoisomeric assignment given in Figure 4C is based on an experiment in which (±)-VX was added to a sample of optically pure (R)-(+)-VX, associated with Eu(hfc)$_3$.

We conclude that association of soman (1, X=O), sarin (2), and tabun (3) with the optically active shift reagents Eu(hfc)$_3$ or Eu(tfc)$_3$ in a molar ratio 1.8-2.5 allows the ready observation of all stereoisomers of these nerve agents in their time-averaged ^{1}H-NMR spectra. The stereoisomers of soman and sarin could be identified in the spectra, using various optically enriched preparations of the agents. Hence, this method of stereochemical analysis of nerve agents is more universally applicable than the gas chromatographic analysis on the Carbowax-Chirasil Val system which only gives satisfactory results for soman. However, at least 0.5 ml of a 1 mM solution of the nerve agent should be available for NMR-analysis of the stereoisomers, which is approximately 10^8 times more than needed for the gas chromatographic analysis of soman using an alkali-flame N/P-detector (Benschop et al. 1981b). Nevertheless, our successful use of C(±)P(+)-soman, isolated after stereospecific inhibition of chymotrypsin by C(±)P(±)-soman for identification of the four stereoisomers of soman, proves that ^{1}H-NMR-analysis of the stereoisomers of nerve agents can be applied e.g. to study in vitro the stereospecificity of their enzymatic reactions.

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