Nerve Agent Stereoisomers: Analysis, Isolation, and Toxicology

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Nerve agents are lethal chemical warfare agents, which were developed a few years before World War II.[1] They were selected for their extreme and acute mammalian toxicity via inhalation or skin penetration as well as for a variety of technological and military tactical reasons.[2] Even if the major world powers agree within the next few years to ban production and stockpiling of these chemicals, nerve agents would remain a major toxicological problem for the next 10–15 years since an estimated 10^5 tons of these extremely hazardous compounds would have to be destroyed after the entry into force of a treaty.[3] Moreover, the confirmed use of chemical agents in the Gulf War[4] without any political repercussions,[5] as well as the presumed proliferation of this type of weapon in Third World countries,[5,6] suggests that efforts to ban chemical warfare may not be completely successful, even if an agreement should be reached between the major powers. Consequently, work on the toxicology and treatment of intoxications with these agents will have to continue. Analytical toxicological studies should provide means for monitoring exposure to agents of workers in destruction facilities and for retrospective detection of nerve agent exposure in case of alleged nonadherence to the treaty.

When nerve agents enter the body a multitude of toxic effects ensues.[7] The acute toxic effects are due to inhibition of acetylcholine hydrolysis by acetylcholinesterase (AChE) caused by phosphorylation of the serine residue in the active site of the enzyme.[8] This results in an uncontrolled increase of acetylcholine concentration at cholinergic synapses, leading to a variety of cholinergic effects such as myosis, salivation, hypotension, bradycardia, muscle tremors, convulsions, and respiratory depression. A fatal outcome of the intoxication is usually due to respiratory failure.[9] Much work has already been done on the biochemistry and pharmacology of the involved processes.[8] This has led to the development of several antidotes for nerve agent intoxications, e.g., anticholinergics such as atropine to antagonize the effects of acetylcholine accumulation at muscarinic receptor sites, pyridinium aldoximes for nucleophilic removal of the organophosphate moiety from AChE, carbamates such as pyridostigmine for prophylaxis by way of partial protection of AChE against inhibition by organophosphates, and diazepam to suppress convulsions and subsequent brain damage.[8,10] Although the combined use of these antidotes will usually lead to survival even after intoxication with doses of nerve agent corresponding to several times the LD_{50}, much work remains to be done, especially with regard to the general condition of the survivor.[11]

In order to examine the conditions affecting survival and the quality of survival, a detailed knowledge of the toxicodynamics and toxicokinetics of nerve agents in mammals is necessary. In order to discuss the relevance of the work that has been done in our research group, the chemical structure of the four major nerve agents (Figure 1) should be considered. Among the relatively volatile nerve agents, *O*-isopropyl methylphosphonofluoridate (sarin) and *O*-(1,2,2-trimethylpropyl) methylphosphonofluoridate (soman) both have fluoride as leaving group for reaction with AChE, whereas *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (tabun) has cyanide as a leaving group. The less volatile agent *O*-ethyl *S*-(2-(diisopropylamino)ethyl) methylphosphonothioate (VX), which penetrates readily through the skin, has a thiocholine type leaving group. A common feature of the four agents is the presence of a stereogenic[12] phosphorus atom, which leads to the presence of equal amounts of enantiomers (pairs of enantiomers for soman) in the synthetic product. In the case of soman four stereoisomers exist, since the

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(1) (a) Robinson, J. P. In *The Problem of Chemical and Biological Warfare: The Rise of CB Weapons*; SIPRI: Almquist and Wiksell; Stockholm, 1971; Vol. 1, pp 71–75. (b) Holmstedt, B. In *Handbuch der experimentellen Pharmakologie. Cholinesterases and Anticholinesterase Agents*; Koelle, G. B., Ed.; Springer: Berlin, 1963; Vol. 15, Chapter 9.

(2) Franke, S. *Lehrbuch der Militärchemie*; Militärverlag der DDR: Berlin, 1977; Vol. 1.

(3) Goldblatt, J. In *SIPRI Yearbook 1987, World Armaments and Disarmaments*; Oxford University: Oxford, 1987; Chapter 11, p 385.

(4) *Report of the Specialists Appointed by the Secretary-General To Investigate Allegations by the Islamic Republic of Iran Concerning the Use of Chemical Weapons*. UN Document S/16433, Mar 26, 1984.

(5) Ember, L. *Chem. Eng. News* **1988**, *66*(13), 7–17.

(6) (a) Robinson, J. P. In *SIPRI Yearbook 1987, World Armaments and Disarmaments*; Oxford University: Oxford, 1987; Chapter 5.VIII, p 103. (b) Ember, L. *Chem. Eng. News* **1986**, *64*(15), 8–16.

(7) Karczmar, A. G. *Fundam. Appl. Toxicol.* **1985**, *5*, S270–S279.

(8) (a) Schumacher, K. *Militärtoxicologie und Militärradiologie*; Militärverlag der DDR: Berlin, 1984. (b) Koelle, G. B. *Handbuch der experimentellen Pharmakologie. Cholinesterases and Anticholinesterase Agents*; Springer: Berlin, 1963; Vol. 15.

(9) Brimblecombe, R. W. *Pharmacol. Ther., Part B* **1977**, *3*, 65–74.

(10) (a) *Proceedings of The Third Symposium on Prophylaxis and Treatment of Chemical Poisoning*, April 22–24, 1985, Stockholm; *Fundam. Appl. Toxicol.* **1985**, *5*(6), Part 2. (b) *Medical Protection against Chemical-Warfare Agents*; SIPRI Books, Almquist and Wiksell, Stockholm. 1976.

(11) Wolthuis, O. L.; Van Wersch, R. A. P.; Van Helden, H. P. M. *Neurobehav. Toxicol. Teratol.* **1986**, *8*, 127–130.

(12) Mislow, K.; Siegel, J. *J. Am. Chem. Soc.* **1984**, *106*, 3319–3328.

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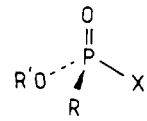

Figure 1. Chemical structure of nerve agents. R = Me, R′ = Pr^i, X = F: (±)-sarin; R = Me, R′ = $Bu^tC(H)Me$, X = F: C(±)P(±)-soman; R = Me_2N, R′ = Et, X = CN: (±)-tabun; R = Me, R′ = Et, X = $SCH_2CH_2N(Pr^i)_2$: (±)-VX.

1,2,2-trimethylpropyl moiety also has a stereogenic center. We distinguish these stereoisomers as C(+)-P(+), C(+)P(−), C(−)P(−), and C(−)P(+), in which C stands for chirality in the 1,2,2-trimethylpropyl moiety. In synthetic C(±)P(±)-soman, the enantiomeric pairs [C(+)P(+) + C(−)P(−)] and [C(+)P(−) + C(−)P(+)] are present in a 45/55 molar ratio.[13]

A priori it should be expected that the stereoisomers of nerve agents have different toxicological properties since they exert their effects in a biological, chiral environment. The first evidence for such stereoselective activity was reported in 1955 by Michel,[14] who observed a biphasic inhibition of AChE upon incubation with an equimolar amount of sarin. Subsequently, Christen[15] succeeded in isolating (−)-sarin via partial stereospecific hydrolysis of (±)-sarin with phosphorylphosphatases in rat plasma, while Boter et al.[16] obtained optically active sarin with <76% ee by way of stereospecific synthesis. Both investigators reported that (−)-sarin is a much more potent inhibitor of AChE than the (+) enantiomer. Early attempts to isolate optically enriched tabun by way of stereoselective enzymatic hydrolysis of (±)-tabun led to inconclusive results.[17,18] Hall and co-workers[19] reported on the anticholinesterase properties of the two enantiomers of VX, which were obtained by straightforward alkylation at the sulfur atom of the resolved salts of *O*-ethyl methylphosphonothioic acid.[20] Attempts to isolate the stereoisomers of nerve agents for use in toxicological investigations were often frustrated by lack of convenient, reliable methods to analyze the resolved isomers. The recent development of versatile gas chromatographic[21] and liquid chromatographic[22] methods for chiral analysis has revived our interest in toxicological research on the stereoisomers of nerve agents. In this Account the following aspects of nerve agent stereoisomerism will be dealt with: (i) chiral analysis, (ii) isolation, (iii) biochemical and toxicological studies, (iv) absolute configuration, and (v) toxicokinetics of soman stereoisomers.

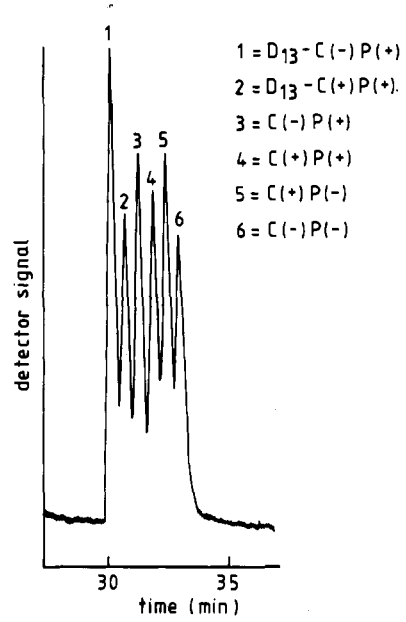

Figure 2. Gas chromatographic resolution of the four stereoisomers of C(±)P(±)-soman and of D_{13}-C(±)P(+)-soman on a 50-m, wide-bore column coated with Chirasil Val.[27]

Chiral Analysis

Because of the volatility of nerve agents and the superior detection methods in gas chromatography, we used chiral gas chromatography for analysis of the nerve agent stereoisomers in preference to chiral liquid chromatography. Almost 10 years ago, capillary Chirasil Val columns for gas chromatography became commercially available.[23] The optically active phase of these columns consists of L-valine *tert*-butylamide bonded to a polysiloxane backbone. This phase was developed by Bayer et al.[24] for gas chromatograpic resolution of amino acid enantiomers, based on diastereoisomeric hydrogen-bonding interactions. At that time we were very interested in resolution of the four stereoisomers of soman, in which fluorine and phosphonyl oxygen are strong acceptors for hydrogen bonds. It was found that indeed Chirasil Val resolved the enantiomeric pairs of soman, although the epimers C(+)P(±)-soman were not resolved. Since Carbowax columns resolve the epimers of C(±)P(±)-soman,[25] such a capillary column was connected in series to the Chirasil Val column. The combined system resolved the four stereoisomers of soman.[26] Subsequently, a quality of Chirasil Val was synthesized in our laboratory that resolves all four stereoisomers of soman (Figure 2)[27] with inverted positions of the two inner peaks relative to the original system. An additional and very valuable quality of Chirasil Val is the resolution of deuteriated soman stereoisomers from nondeuteriated analogues. When all hydrogens in the 1,2,2-trimethylpropyl moiety of soman are replaced by deuterium, the P(+) isomers of

(13) Benschop, H. P.; Konings, C. A. G.; Van Genderen, J.; De Jong, L. P. A. *Toxicol. Appl. Pharmacol.* **1984**, *72*, 61–74.
(14) Michel, H. O. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1955**, *14*, 255.
(15) Christen, P. J.; Van Den Muysenberg, J. A. C. M. *Biochim. Biophys. Acta* **1965**, *110*, 217–220.
(16) Boter, H. L.; Ooms, A. J. J.; Van Den Berg, G. R.; Van Dijk, C. *Recl. Trav. Chim. Pays-Bas* **1966**, *85*, 147–150.
(17) Hoskin, F. C. G.; Trick, G. S. *Can. J. Biochem. Physiol.* **1955**, *33*, 963–969.
(18) Augustinsson, K.-B. *Acta Chem. Scand.* **1957**, *11*, 1371–1377.
(19) Hall, C. R.; Inch, T. D.; Inns, R. H.; Muir, A. W.; Sellers, D. J.; Smith, A. P. *J. Pharm. Pharmacol.* **1977**, *29*, 574–576.
(20) De Jong, L. P. A.; Benschop, H. P. In *Stereoselectivity of Pesticides: Biological and Chemical Problems. Chemicals in Agriculture*; Ariens, E. J., Van Rensen, J. J. F., Welling, W., Eds.; Elsevier: Amsterdam, 1988; Vol. 1, Chapter 4.
(21) (a) Schurig, V. *Kontakte (Darmstadt)* **1986**, *1*, 3–22. (b) König, W. A. *The Practice of Enantiomer Separation by Capillary Gas Chromatography*; Hüthig: Heidelberg, 1987. (c) Souter, R. W. *Chromatographic Separation of Stereoisomers*; CRC: Boca Raton, FL, 1987; Chapter 2.
(22) (a) Hara, S.; Gazes, J. *J. Liq. Chromatogr.* **1986**, *9*, 241–694. (b) Souter, R. W. *Chromatographic Separation of Stereoisomers*; CRC: Boca Raton, FL, 1987; Chapter 3.
(23) Frank, H.; Nicholson, G. J.; Bayer, E. *Angew. Chem.* **1978**, *90*, 396–398.
(24) Bayer, E.; Frank, H. In *Modification of Polymers*; ACS Symposium Series 121; American Chemical Society; Washington, DC, 1980; pp 341–358.
(25) Verweij, A.; Burghardt, E.; Koonings, A. W. *J. Chromatogr.* **1971**, *54*, 151–156.
(26) Benschop, H. P.; Konings, C. A. G.; De Jong, L. P. A. *J. Am. Chem. Soc.* **1981**, *103*, 4260–4262.
(27) Benschop, H. P.; Bijleveld, E. C.; Otto, M. F.; Degenhardt, C. E. A. M.; Van Helden, H. P. M.; De Jong, L. P. A. *Anal. Biochem.* **1985**, *151*, 242–253.

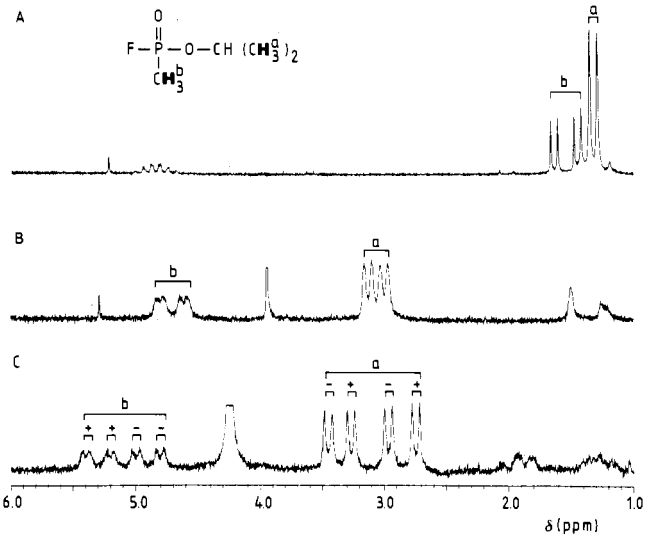

Figure 3. ^1H NMR spectra (100 MHz) of (±)-sarin (14.5 mM) in $CCl_4/CDCl_3$ (3/1 (v/v)): (A) no shift reagent added; (B) achiral tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium(III) [Eu(fod)$_3$] added (7.0 mM); (C) optically active tris[(1*R*)-3-((heptafluorobutyryl)camphorato)]europium(III) [Eu(hfc)$_3$] added (6.9 mM).[29]

the D_{13}-analogue are completely separated from the four stereoisomers of soman, as shown in Figure 2 (vide infra for peak identification). Therefore, D_{13}-C(±)P(+)-soman can be used as an isotopically and stereochemically labeled internal standard to analyze soman stereoisomers in, e.g., biological samples, without the need to use a costly GC/MS combination.[27]

Whereas Chirasil Val columns give a very incomplete resolution of the two enantiomers of sarin, tabun and VX are not resolved at all. For the analytical resolution of these enantiomeric pairs, the strong, reversible association of phosphoryl oxygen with lanthanide ions in optically active camphorate salts was exploited for chiral NMR analysis.[28] This approach permitted the analysis at a millimolar level of all stereoisomers of sarin, soman, tabun, and VX.[29] The ^1H NMR spectra of (±)-sarin in the absence of a shift reagent (Figure 3A), in the presence of the achiral shift reagent Eu(fod)$_3$ (Figure 3B), and in the presence of the optically active shift reagent Eu(hfc)$_3$ (Figure 3C) are given as examples. The two diastereoisotopic methyl groups of the isopropyl moiety of sarin become nonequivalent on the NMR time scale in the presence of Eu(fod)$_3$. When Eu(fod)$_3$ is replaced by Eu(hfc)$_3$, the two enantiomers of sarin become visible since the sets of CH_3–C hydrogen doublets split up once more. Although less clearly, the enantiomers also become visible in the two sets of quartets of the CH_3–P hydrogens (Figure 3C).

Since gas chromatographic analysis allows a 10^6-fold lower detection limit than NMR, we have also investigated the gas chromatographic equivalent of chiral shift NMR, i.e., chiral complexation gas chromatography with optically active bis[(1*R*)-3-((heptafluorobutyryl)camphorato)]nickel(II) phase, as developed by Schurig and co-workers.[21] On a wide-bore column coated with 6% of the nickel compound in a methylsilicone phase, Degenhardt and co-workers in our laboratory[30] resolved the enantiomeric pairs of sarin and tabun as well as the four stereoisomers of soman (Figure 4). VX, however, could not be resolved with this approach.

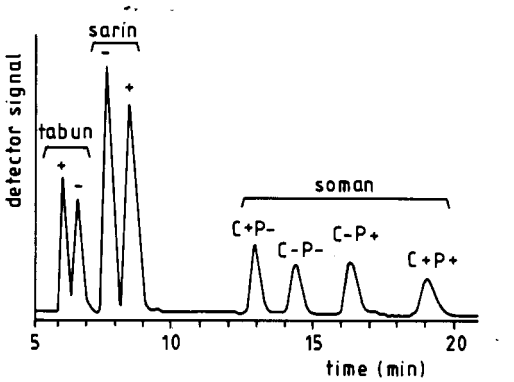

Figure 4. Gas chromatogram obtained after injection of (±)-tabun, (±)-sarin, and C(±)P(±)-soman on an 11-m, wide-bore column coated with bis[(1*R*)-3-((heptafluorobutyryl)camphorato)]nickel(II) in OV-101.[30]

In practice, the two chiral gas chromatographic methods as well as the chiral NMR method proved to complement each other for analysis of nerve agent stereoisomers. Therefore, these three approaches have been used in all subsequent investigations.

Isolation

The above-mentioned analytical procedures were used for monitoring our attempts to isolate the nerve agent stereoisomers. In order to obtain these isomers on a 1-20-mg scale for further investigations, we used stereoselective enzymatic reactions in combination with classical resolution methods and stereoselective syntheses. The isolation of the four stereoisomers of C(±)P(±)-soman is a representative example.[13] A partial separation is first achieved via complete resolution of the two enantiomers of 3,3-dimethyl-2-butanol, from which C(+)P(±)- and C(−)P(±)-soman are synthesized. Each of the two pairs of epimers is incubated with α-chymotrypsin in aqueous solution. Under optimal conditions of pH, temperature, ionic strength, concentration, and ratio of reactants, the enzyme is inhibited by virtually all P(−)-soman in the solution. The C(+)P(+) and C(−)P(+) isomers are isolated with optical purities >99% and with appropriate optical rotations in yields of 20–30%.

Conversely, the plasma of rabbits and of many other species contains a group of enzymes named phosphorylphosphatases,[31] which hydrolyze the phosphorus–cyanide or phosphorus–fluorine bond of nerve agents. For the latter bond, the stereoselectivity of the hydrolysis reaction is opposite to that of the inhibition reaction of α-chymotrypsin. Therefore, after a 1-min incubation of C(−)P(±)- and C(+)P(±)-soman in rabbit plasma, the optically pure C(−)P(−) and C(+)P(−) isomers, respectively, can be isolated in ca. 20% yield. Solutions of the soman isomers in solvents such as ethyl acetate are optically stable upon storage at −25 °C, or even at room temperature, for several months provided that the concentrations of the isomers are ≤1 mM. By

(28) Sullivan, G. R. In *Topics in Stereochemistry*; Eliel, E. L., Allinger, N. L., Eds.; Wiley: New York, 1978; Vol. 10, pp 287–329.
(29) Van Den Berg, G. R.; Beck, H. C.; Benschop, H. P. *Bull. Environ. Contam. Toxicol.* **1984**, *33*, 505–514.
(30) Degenhardt, C. E. A. M.; Van Den Berg, G. R.; De Jong, L. P. A.; Benschop, H. P.; Van Genderen, J.; Van De Meent, D. *J. Am. Chem. Soc.* **1986**, *108*, 8290–8291.
(31) Mounter, L. A. In *Handbuch der experimentellen Pharmakologie. Cholinesterases and Anticholinesterase Agents*; Koelle, G. B., Ed.; Springer: Berlin, 1963; Vol. 15, Chapter 10.

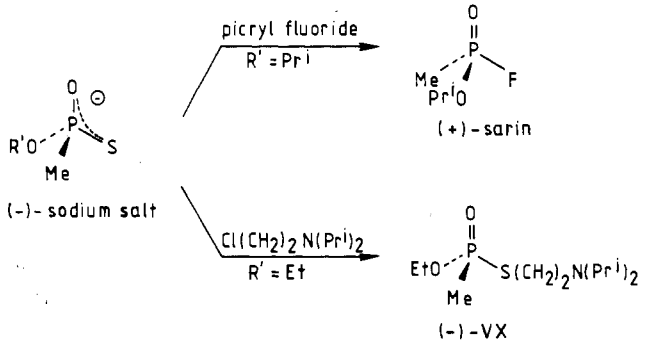

Figure 5. Stereoselective synthesis of (+)-sarin[14] and of (-)-VX.[19]

analogy with soman, the optically pure (-) isomer of sarin was obtained by hydrolysis of the (+) isomer in (±)-sarin by rabbit plasma.[32] Apart from AChE, which is too costly to be used in large quantities, we have not found an enzyme that removes (-)-sarin with any significant stereoselectivity from (±)-sarin. At present, the only methods to obtain optically enriched (+)-sarin with 40–75% ee is either by means of a stereoselective conversion of the sodium salt of (-)-*O*-isopropyl methylphosphonothioic acid with picryl fluoride, predominantly with inversion of configuration (Figure 5),[16] or by means of stereoselective phosphonylation of α-cyclodextrin[32,33] with (±)-sarin in aqueous solution at pH 9.0.

In the case of (±)-tabun, incubation with α-chymotrypsin leaves the optically pure (-) isomer in solution, which can then be isolated with ≥98% ee.[30] Surprisingly, the stereoselectivity of hydrolysis of tabun enantiomers by phosphorylphosphatases appears to be species dependent. For example, incubation in plasma from horses and cows produces tabun enriched with the (+) isomer, but incubation with plasma from mice, sheep, rabbits, guinea pigs, pigs, and humans produces tabun with a moderate excess of the (-) isomer. Incubation in rat plasma appears to be the only method that produces (+)-tabun with satisfactory (92–99%) optical purity.[30]

Finally, the two enantiomers of VX can be obtained almost optically pure on a gram or even much larger scale by straightforward alkylation at sulfur in the resolved enantiomers of *O*-ethyl methylphosphonothioic acid (Figure 5).[19]

Biochemical and Toxicological Studies

Obviously, one of the primary reasons to obtain the separate stereoisomers of nerve agents was to identify the peaks in gas chromatograms and NMR spectra that have been dealt with in preceding paragraphs. A further reason to isolate the nerve agent stereoisomers as optically pure as possible on a milligram scale is to investigate their acute toxicity. A priori it should be expected that the lethal effects of the stereoisomers correlate with their inhibitory potency toward AChE. Therefore, we measured the bimolecular rate constants of inhibition of AChE with the stereoisomers, as well as their LD_{50} values in mice. It appeared that two groups of nerve agents can be distinguished (Table I). The first group consists of soman and sarin, for which

Table I
Stereoselectivity in Anticholinesterase Activity and Acute Lethality of Nerve Agent Stereoisomers

nerve agent stereoisomer	rate constant for inhibition of AChE,[a] M^{-1} min^{-1} (25 °C)	LD_{50} (mouse), μg/kg	ref
C(+)P(-)-soman	2.8×10^8	99[b]	13
C(-)P(-)-soman	1.8×10^8	38[b]	13
C(+)P(+)-soman	$<5 \times 10^3$	>5000[b]	13
C(-)P(+)-soman	$<5 \times 10^3$	>2000[b]	13
C(±)P(±)-soman		156[b]	13
(-)-sarin	1.4×10^7	41[c]	34, 35
(+)-sarin	$<3 \times 10^3$[d]		34
(±)-sarin		83[c]	35
(-)-tabun	2.3×10^6	119[c]	30
(+)-tabun	3.7×10^5	837[c]	30
(±)-tabun		208[c]	30
(-)-VX	4×10^8	12.6[c]	35, 19
(+)-VX	2×10^6	165[c]	35, 19
(±)-VX		20.1[c]	35

[a] Rate constants for soman and tabun isomers were measured with AChE from electric eel at pH 7.5, whereas those for sarin and VX enantiomers were obtained with AChE from bovine erythrocytes at pH 7.7. [b] Subcutaneous administration. [c] Intravenous administration. [d] Estimated from an experiment with optically enriched (+)-sarin (64% ee).[34]

the rates of inhibition of AChE by the P(+) isomers are 3–4 orders of magnitude less than those by the P(-) isomers.[13,34] Concomitantly, it was found that the LD_{50} values of the P(-) isomers of soman are much lower than those of the P(+) isomers.[13] The difference is such that one may state that the C(±)P(+) isomers are present in C(±)P(±)-soman as nontoxic components. We predict a similar situation for (±)-sarin, but as mentioned before, we have not yet been able to isolate (+)-sarin of sufficient optical purity to determine its LD_{50}. The second group of nerve agents consists of tabun and VX, for which the rates of inhibition of AChE by the (+) and (-) enantiomers differ by only 1–2 orders of magnitude.[30,35] For both agents, the rapidly inhibiting isomers also have a lower LD_{50}, but the differences are less dramatic than in the case of the soman stereoisomers.

In general, the P(-)/P(+) ratios for inhibition of AChE by nerve agent enantiomers indicate the relative degree of their acute toxicities. However, it should be stressed that small differences in anticholinesterase activity, such as between C(+)P(-)- and C(-)P(-)-soman (Table I), do not alone determine relative acute toxicities. Such minor differences are easily overruled[13] by opposite stereoselectivities of other in vivo interactions of the stereoisomers. We have not yet systematically investigated synergism and antagonism in the acute lethality of nerve agent stereoisomers. Our data (Table I), however, do not suggest that such effects will be important, since the LD_{50} values of the P(-) isomers are approximately half that of the mixture of P(±) isomers.

In cooperation with other research groups, further toxicological properties of the soman stereoisomers have been investigated. With Brimfield et al.[36] we measured the affinity of the soman stereoisomers for a monoclonal antibody raised against C(±)P(±)-soman. The antibody

(32) Van Den Berg, G. R.; Benschop, H. P., Prins Maurits Laboratory TNO, Rijswijk, The Netherlands, unpublished results, 1987.
(33) Van Hooidonk, C.; Breebaart-Hansen, J. C. A. E. *Recl. Trav. Chim. Pays-Bas* **1970**, *89*, 289–299.
(34) Boter, H. L.; Van Dijk, C. *Biochem. Pharmacol.* **1969**, *18*, 2403–2407.
(35) Van De Meent, D.; Van Genderen, J., Medical Biological Laboratory TNO; Van Den Berg, G. R., Prins Maurits Laboratory TNO, Rijswijk, The Netherlands, unpublished results, 1987.
(36) Brimfield, A. A.; Hunter, K. W.; Lenz, D. E.; Benschop, H. P.; Van Dijk, C.; De Jong, L. P. A. *Mol. Pharmacol.* **1985**, *28*, 32–39.

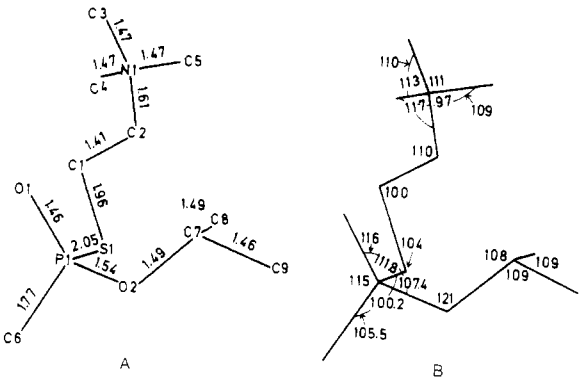

Figure 6. Conformation, bond lengths (A), and bond angles (B) of (*R*)-(+)-*O*-isopropyl *S*-(2-(trimethylammonio)ethyl) methylphosphonothioate iodide.[40]

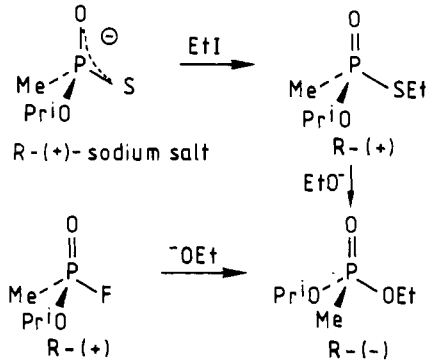

Figure 7. Chemical correlation of the absolute configuration of (+)-sarin with that of (*R*)-(+)-*O*-isopropyl methylphosphonothioic acid (sodium salt).

showed a slightly higher affinity for the C(±)P(−) isomers than for the C(±)P(+) isomers, with affinity constants (pH 7.4, 25 °C) varying between 5.9×10^5 M^{-1} for the C(+)P(−) isomer and 5.2×10^4 M^{-1} for C(+)-P(+)-soman. Clement et al.[37] found that the K_i values for inhibition of mouse serum aliesterase are about 2 orders of magnitude higher for the C(±)P(+) than for the C(±)P(−) isomers. Conversely, Johnson et al.[38,39] concluded that the C(±)P(+) isomers inhibit neuropathy target esterase from hen brains more rapidly than the C(±)P(−) isomers.

Absolute Configuration

The absolute configurations at phosphorus of the stereoisomers of nerve agents are not yet firmly established. However, an X-ray analysis has been performed[40] on a single crystal of the dextrorotatory enantiomer of a quaternized isopropoxy analogue of VX, i.e., *O*-isopropyl *S*-(2-(trimethylammonio)ethyl) methylphosphonothioate iodide, $Pr^iO(Me)P(O)S(CH_2)_2N^+(Me)_3I^-$. This established the (*R*) configuration of the enantiomer and of (+)-*O*-isopropyl methylphosphonothioic acid, from which the inhibitor is obtained by alkylation of sulfur (cf. Figure 5) and subsequent quaternization of nitrogen. The conformation, bond lengths, and bond angles of the molecule

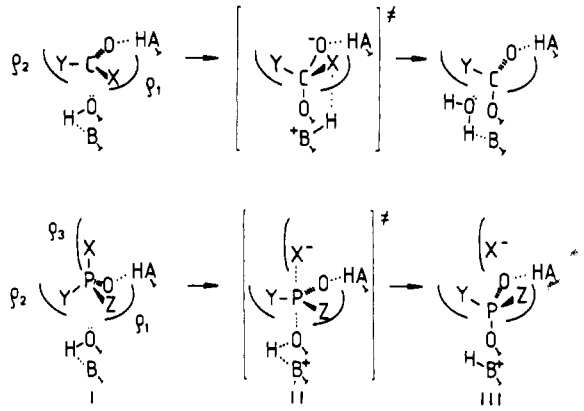

Figure 8. Schematic representation of acetylcholine hydrolysis and inhibition by organophosphates at the active site of AChE according to Järv.[20,46] See text for further explanation.

are shown in Figure 6.[41] Similar to (+)-VX,[19,20] the (*R*)-(+) enantiomer is the least potent AChE inhibiting[42] and the least toxic[43] of the two enantiomers.

Figure 7 shows the chemical correlation of the configuration of (*R*)-(+)-*O*-isopropyl methylphosphonothioic acid with that of (+)-sarin.[16] Since alkylation at sulfur proceeds with retention, whereas the nucleophilic displacements of the thioethyl substituent and of fluorine by ethoxy probably proceed with inversion of configuration,[44] it follows that the absolute configuration of (+)-sarin is, probably, *R*.

In general, it appears that for a wide variety of leaving groups (e.g., fluoride, $-SCH_2CH_2N^+Me_3$, and *p*-nitrophenolate), the enantiomer with the absolute configuration as shown in Figure 1 is the most active AChE inhibitor, provided that R′O is bulkier than R.[45] This regularity in the stereospecificity can be rationalized with the model developed by Järv[20,46] for the active site of AChE (Figure 8). In addition to two catalytic sites, i.e., a basic group activating the serine hydroxyl group and an acidic group involved in hydrogen bonding with the carbonyl oxygen of the substrate or with the phosphoryl oxygen of the inhibitor, this model features two hydrophobic subsites ρ_1 and ρ_2, for binding of the alcohol and acyl moieties of the substrate $R_1C(O)OR_2$, respectively. Structure–activity studies with substrates have shown that the size of ρ_2 is rather limited, accommodating only acetyl groups, whereas ρ_1 can bind up to butoxy groups. During inhibition with organophosphate, it is assumed that the leaving group X is in an axial position of the trigonal-bipyramidal transition state, opposite to the axially entering serine hydroxyl group,[47] with the position of phosphoryl oxygen fixed via hydrogen bonding. Consequently, the groups R and OR′ should be located at the subsites ρ_1 and ρ_2. This explains the preferential interaction of AChE with one

(37) Clement, J. G.; Benschop, H. P.; De Jong, L. P. A.; Wolthuis, O. L. *Toxicol. Appl. Pharmacol.* **1987**, *89*, 141–143.
(38) Johnson, M. K.; Read, D. J.; Benschop, H. P. *Biochem. Pharmacol.* **1985**, *34*, 1945–1951.
(39) Johnson, M. K.; Willems, J. L.; De Bisschop, H. C.; Read, D. J.; Benschop, H. P. *Toxicol. Appl. Pharmacol.* **1988**, *92*, 34–41.
(40) Mehlsen-Sørensen, A. *Acta Crystallogr., Sect. B* **1977**, *B33*, 2693–2695.
(41) Interestingly, the dihedral angle of the thiocholine group in this isomer is 171°, similar to the antiperiplanar conformation of acetyl- and propionylthiocholine, which are substrates for AChE. See: Shefter, E.; Mautner, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 1253–1260.
(42) De Jong, L. P. A.; Van Dijk, C. *Biochim. Biophys. Acta* **1972**, *268*, 680–689.
(43) Benschop, H. P.; Konings, C. A. G.; Van Genderen, J.; De Jong, L. P. A. *Fundam. Appl. Toxicol.* **1984**, S84–S95.
(44) Hall, C. R.; Inch, T. D. *Tetrahedron* **1980**, *36*, 2059–2095.
(45) Benschop, H. P. *Pestic. Biochem. Physiol.* **1975**, *5*, 348–349.
(46) Järv, J. *Bioorg. Chem.* **1984**, *12*, 259–278.
(47) In a further refinement of the model, Järv[46] assumes a third subsite ρ_3, which interacts only with the leaving group of organophosphate inhibitors, accommodating leaving groups with a size up to R = *n*-octyl in inhibitors of the general type $(EtO)_2P(O)SR$.

of the enantiomers of an organophosphate in which R and OR′ (Figure 1) differ considerably in bulkiness with one of these groups having preferentially the size of only a methyl group (e.g., as in sarin and soman). Conversely, the model also accommodates the less distinct stereospecificity in the inhibition of AChE by tabun and VX, in which the groups R and OR′ differ less in bulkiness than in sarin and soman.

Toxicokinetics of Soman Stereoisomers

The integration of chiral analytical techniques with toxicology has proven to be particularly useful for our recent investigations of the toxicokinetics of C(±)-P(±)-soman in rats.[48] Such studies deal with the in vivo distribution and elimination of soman stereoisomers as a function of dose and time after administration. Until recently, it was considered irrelevant to study the toxicokinetics of phosphofluoridates and of tabun since these agents were supposedly "hit and run" poisons with an extremely rapid in vivo breakdown. A few years ago Wolthuis and co-workers at the Medical Biological Laboratory TNO found that rats challenged with a dose of 6 × LD_{50} of C(±)P(±)-soman could be kept alive by immediate treatment with atropine and newly developed reactivators of soman-inhibited AChE. However, the animals became reintoxicated and died 4–6 h after this initially successful treatment.[49] This result suggested that C(±)P(±)-soman is much more persistent in vivo than previously thought. In order to measure the presumably very low in vivo levels of soman isomers, we developed an analytical procedure[27,48] that included stabilization of the soman isomer levels in blood samples as a crucial factor. The soman isomers and internal standard, i.e., D_{13}-C(±)P(+)-soman (vide supra), are extracted from the stabilized sample into ethyl acetate and analyzed on the Chirasil Val column. With the combined use of thermodesorption/cold trap injection of large sample volumes and alkali flame detection, a minimum detectable concentration in blood of ca. 8 pM per soman isomer could be verified. The latter minimum detectable concentration is close to the lower limit of toxicological significance,[48] i.e., sufficient for toxicokinetic investigations. These studies were performed in rats at intravenous doses of 0.5 and 0.25 mg/kg, corresponding to 6 × LD_{50} and 3 × LD_{50} of C(±)P(±)-soman, respectively. The first blood sample was taken 0.25 min after administration of C(±)-P(±)-soman to the anesthetized, atropinized, and artificially ventilated animals. Even at that early time point, the C(+)P(+) isomer had disappeared from the blood. The other P(+) isomer, i.e., C(−)P(+)-soman, could be followed up to 4 min after administration of C(±)P(±)-soman. Evidently, both relatively nontoxic P(+) isomers are extremely unstable in vivo, presumably due to rapid hydrolysis by phosphorylphosphatases. In contrast (Figure 9), the extremely toxic P(−) isomers could be followed in rat blood for at least 4 h at a dose of 6 × LD_{50} and for 3 h at a dose of 3 × LD_{50} before the blood levels dropped below 10–20 pg/mL for each P(−) isomer.

A toxicokinetic model for the C(±)P(−) isomers of soman involves (i) distribution from the central compartment to two peripheral compartments in order to accommodate the observed three-exponential decay of C(±)P(−)-soman blood levels, (ii) a rapid initial elimination by covalent phosphorylation reactions in all compartments, and (iii) a more gradual further elimination by enzymatic and spontaneous hydrolysis, also in all compartments.[48]

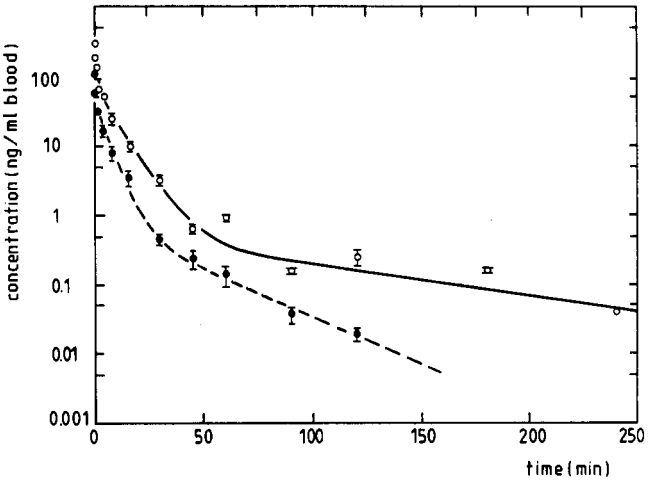

Figure 9. Decay of the concentrations (±s.e.m.) of C(+)P(−)-soman with time in blood of anesthetized, atropinized, and artificially ventilated rats after intravenous administration of 6 × LD_{50} (495 μg/kg) (○) and 3 × LD_{50} (●) of C(±)P(±)-soman.[48]

Measurement of blood levels of soman isomers as a function of time and the ensuing toxicokinetic model will help considerably to put the toxicology of soman, as well as the treatment of intoxication by this agent, on a quantitative basis. For example, the earlier mentioned reintoxication phenomena in rats at a dose of 6 × LD_{50} were not observed at a dose of 3 × LD_{50}. This result can now be understood on the basis of the substantial decrease in the terminal half-lives of C(±)-P(−)-soman when the dose is halved, i.e., from 40–60 to 15–20 min (Figure 9). It can be calculated that the soman isomers are eliminated too quickly at the lower dose to reinhibit oxime-reactivated AChE to a significant degree.[48]

Concluding Remarks

Biochemical and toxicological studies with chiral nerve agents have demonstrated that it is essential to differentiate between the various stereoisomers in view of their widely diverging properties. The rapid developments in the field of enantiomer analysis allowed us to adapt two chiral gas chromatographic methods and a chiral NMR method for such a differentiation. These procedures have been used to monitor the isolation of the stereoisomers of soman, tabun, and VX, as well as (−)-sarin on a milligram scale with better than 98% ee. Therefore, the methodology is now available for future studies in which the various nerve agent stereoisomers are taken into account.

Advantage should be taken of our toxicokinetic investigations of nerve agent stereoisomers as a quantitative basis for modeling of prophylaxis and therapy of poisoning with these agents and to improve the rational development of new antidotes.

Acute lethality determinations were made by John Van Genderen and Dory Van De Meent, and animal toxicokinetic experiments were performed by Dr. Herman Van Helden and

(48) Benschop, H. P.; Bijleveld, E. C.; De Jong, L. P. A.; Van der Wiel, H. J.; Van Helden, H. P. M. *Toxicol. Appl. Pharmacol.* **1987**, *90*, 490–500.
(49) Wolthuis, O. L.; Berends, F.; Meeter, E. *Fundam. Appl. Toxicol.* **1981**, *1*, 183–195.

Herma Van Der Wiel in the Department of Toxicology (Head Dr. Otto Wolthuis) of the Medical Biological Laboratory TNO. Dr. Henk Boter and George Van Den Berg, Prins Maurits Laboratory TNO, developed the stereoselective syntheses of sarin and VX, and Carla Degenhardt from the same laboratory contributed to the development of the chiral gas chromatography. Financial support of our toxicokinetic investigations by the U.S. Army Medical Research and Development Command under Grants DAMD17-85-G-5004 and DAMD17-87-G-7015 is gratefully acknowledged.

Transformations of Carbon Monoxide and Related Ligands on Metal Ensembles

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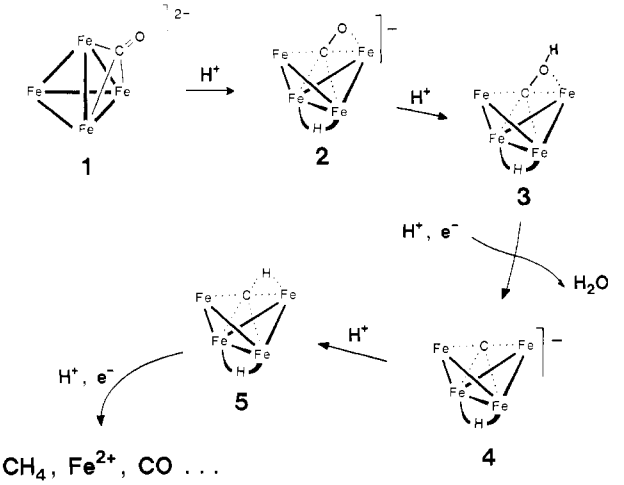
The reactions of ligands coordinated to metal atoms have enormous importance in disciplines ranging from bioinorganic chemistry[1,2] to organometallic chemistry[3] and applications ranging from industrial homogeneous catalysis[4,5] to inorganic and organic syntheses.[6,7] Although the majority of these reactions occur on single metal centers, there is increasing interest in the reactivity of ligands attached to metal clusters. The presence of several metals in proximity to a ligand may result in patterns of ligand stabilization and reactivity that have not been observed for monometal complexes. In this Account, we describe the role of the metal cluster in converting carbon monoxide to C and CCO ligands and the influence of the cluster on the reactions of these ligands. This chemistry provides many examples of facile C–O, C–C, and C–H bond formation and cleavage and insight into the ways metal ensembles influence these reactions.

Aside from its tendency to coordinate metal atoms in low oxidation states and to be attacked by electrophiles, carbon monoxide is a relatively inert molecule. However, the reactions of CO are promoted by heterogeneous or homogeneous metal catalysts; examples include the oxidation of CO in automobile exhausts,[8,9] the incorporation of CO into olefins in the hydroformylation reaction,[10] the reduction of CO to methanol,[10,11] and the conversion of CO to hydrocarbons in the Fischer–Tropsch process.[12] In each of these catalytic processes, an early step in the reaction sequence appears to be the coordination of carbon monoxide to a metal atom or metal particle. The first three processes appear to occur without CO cleavage, whereas in the Fischer–Tropsch process, CO cleavage apparently occurs to produce an intermediate surface carbide.[13,14] In a pioneering study of the conversion of carbon monoxide to methane on copper–nickel alloys, Araki and Ponec found that an ensemble of several nickel atoms is required to effect the critical CO cleavage process.[14] The details of this cleavage reaction on nickel are obscure because structural information on ligand–metal bonding is difficult to obtain on surfaces. By contrast, transformations in

Scheme I



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Michael J. Sailor received his B.S. degree from Harvey Mudd College in 1983, where he did undergraduate research with Mitsuru Kubota. He received his Ph.D. from Northwestern University in 1987 for research on ruthenium and osmium ketenylidenes. At present, he is pursuing photoelectrochemistry as a postdoctoral fellow with Nathan Lewis at Caltech.

(1) Spiro, T. G., Ed. *Metal Ions in Biology, Zinc Enzymes*; Wiley: New York, 1980.
(2) Spiro, T. G., Ed. *Metal Ions in Biology, Metal Ion Activation of Dioxygen*: Wiley: New York, 1980.
(3) Collman, J. P.; Hegedus, L. S.; Norton, J. R.; Finke, R. G. *Principles and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1987.
(4) Parshall, G. W. *Homogeneous Catalysis*: Wiley-Interscience: New York, 1980.
(5) Kochi, J. K. *Organometallic Mechanisms and Catalysis*; Academic: New York, 1978.
(6) Hipp, C. J.; Busch, D. H. In *Coordination Chemistry*; *ACS Monograph* 175; Martell, A. E., Ed.; American Chemical Society: Washington, DC, 1978; Vol. 2.
(7) Hartley, F. R.; Patai, S., Eds. *The Chemistry of Functional Groups, The Chemistry of Metal–Carbon and Carbon–Carbon Bond Forming Reactions Using Organometallic Compounds*; Wiley-Interscience: New York, 1985.
(8) Engel, T.; Ertl, G. *Adv. Catal.* **1979**, *28*, 1.
(9) Taylor, K. C. *Catal. Sci. Technol.* **1984**, *5*, 119.
(10) Sheldon, R. A. *Chemicals from Synthesis Gas, Catalytic Reactions of CO and H_2*; Reidel: Boston, 1983.
(11) Falbe, J., Ed. *New Syntheses with Carbon Monoxide*: Springer: Berlin, 1980.
(12) Anderson, R. B. *The Fischer–Tropsch Synthesis*; Academic: Orlando, 1987.
(13) Biloen, P.; Sachtler, W. M. H. *Adv. Catal.* **1981**, *30*, 165.
(14) Araki, M.; Ponec, V. *J. Catal.* **1976**, *44*, 439.

0001-4842/88/0121-0374$01.50/0