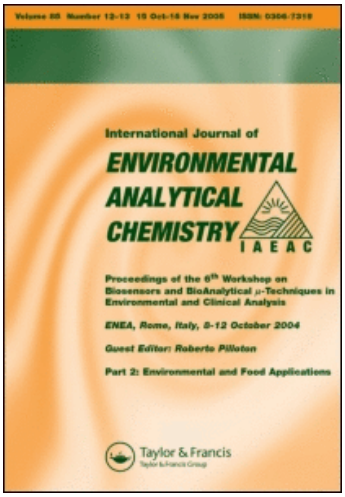

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Assay of the Chiral Organophosphate, Soman, in Biological Samples†

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The anticholinesterase, soman, $(CH_3)_3CC(H)CH_3O(CH_3)P(O)F$, consists of four stereoisomers assigned as C(±)P(±)-soman in which C stands for chirality in the pinacolyl moiety and P for chirality at phosphorus. The four stereoisomers are separated by gas chromatography on an optically active Chirasil–Val column, synthesized and coated in house, or on a Chirasil–Val column identical with the commercially available column when combined with a Carbowax 20M column. This method in combination with an assay based on acetylcholinesterase inhibition shows that the two isomers which do not have anticholinesterase activity, i.e. C(±)P(+)-soman, are rapidly degraded in rat blood due to hydrolysis by phosphoryl-phosphatases. Epimeric soman isomers, e.g. C(±)P(−)-soman, can be separately assayed on a Carbowax or a CPSil 8 column, using ^{2}H-labeled soman isomers as internal standards. ^{2}H-labeled soman stereoisomers serve as internal standards in GC-assay of all four stereoisomers on Chirasil–Val.

For work-up of the four stereoisomers from rat blood the sample is first stabilized by (i) acidification to pH 4.2 at 0°C to suppress hydrolysis by phosphoryl-phosphatases, (ii) addition of aluminum ions for complexation of fluoride ions to prevent regeneration of C(±)P(−)-soman by free fluoride ions from soman-inhibited carboxylesterase, and (iii) addition of $(CH_3)_3CCH_2O(CH_3)P(O)F$ to occupy covalent binding sites for C(±)P(−)-soman, before extraction with a Sep-Pak C_{18} cartridge and elution with ethyl acetate. Using a splitless or on-column injection technique and

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alkali flame ionization detection, the minimum detectable concentration is 30 pg/3-ml blood sample.

KEY WORDS: Soman, stereoisomers, gas chromatography, metabolism, rat blood.

INTRODUCTION

The toxicity of the nerve agent, soman, $(CH_3)_3CC(H)CH_3O(CH_3)P(O)F$ (see Figure 1), is mainly based on its high inhibitory potency for acetylcholinesterase (AChE). Treatment of soman intoxication with the standard antidotes against anticholinesterases, atropine and an oxime, is hampered by rapid conversion of soman-inhibited AChE into a form which can no longer be reactivated by oxime (aging).[1,2] In addition, evidence has accumulated for a much longer *in vivo* persistence of the agent than previously assumed, presenting further difficulties in medical treatment of soman intoxications.[3] Toxicokinetic studies for assessment of the role of intact soman in the body require methods for analysis of trace amounts of this highly toxic agent. Such studies are complicated by the presence of two chiral centers in the soman molecule leading to four stereoisomers designated as C(+)P(+), C(+)P(−), C(−)P(+) and C(−)P(−) in which C and P stand for the two chiral centers (Figure 1). These isomers may differ in inhibitory potency towards AChE[4] and other esterases,[5] acute toxicity,[6] rate of detoxification,[7,8] rate of passage through

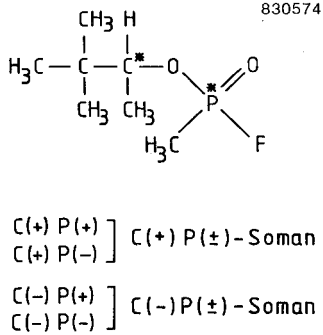


Figure 1 The four stereoisomers of C(±)P(±)-soman. The chiral centers are denoted by an asterisk.

membranes, reversible binding, etc. Analysis methods should, therefore, distinguish between the four stereoisomers.

In this paper studies will be described in which GC-analysis methods were developed for this purpose. In addition, the paper deals with a study on the development of a work-up procedure of the four stereoisomers from rat blood samples in toxicokinetic studies.

MATERIALS AND METHODS

Materials

Electric eel acetylcholinesterase (AChE, EC 3.1.1.7) and bovine pancreas chymotrypsin (EC 3.4.21.1) were procured from Sigma Chemical Co., St. Louis, U.S.A., as preparations type V-S or VI-S and preparation type II, respectively.

The organophosphates† were prepared in this laboratory according to standard procedures. (+)- and (−)-pinacolyl alcohol, starting compounds for synthesis of C(+)P(±)-soman and C(−)P(±)- and D_3-C(−)P(±)-soman, were obtained by resolution of the alcohol as described by Benschop *et al.*[9] The deuterated starting compounds for the synthesis of D_{13}- and D_3-soman, D_{13}-pinacolyl alcohol and D_3-methylphosphonic dichloride and difluoride, respectively, were prepared from D_6-acetone and D_3-methyl iodide, respectively.[10] The single stereoisomers of soman as well as D_3-C(−)P(+)-soman and D_{13}-C(±)P(+)-soman were isolated after treatment of the appropriate preparation with chymotrypsin (P(+)-isomers) or rabbit serum (P(−)-isomers) according to Benschop *et al.*[9,10] The synthesis of Chirasil–Val was performed according to Bayer and Frank[11–13] except for the final condensation step of Chirasil with L-valine tert.-butylamide which was performed with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline instead of N,N′-dicyclohexylcarbodiimide.[10] All other chemicals were obtained commercially.

†Warning: In view of their extreme toxicities, the organophosphates should be handled only in specialized laboratories, where trained medical personel is continuously present.

Coating of capillary columns

Capillaries drawn from Duran 50 glass were roughened by deposition of NaCl and coated dynamically with a 25% solution of Chirasil–Val in *n*-pentane or a solution of Carbowax 20M in methylene chloride by using the mercury plug method.[10,14,15]

Gas chromatography

A gas chromatograph (Packard model 428, Carlo Erba 5160, Pye-104 or Perkin-Elmer Sigma 3) equipped with an alkali flame ionization detector or a FID-detector was used. The following glass capillary columns were applied: type I Chirasil–Val[10] (coated in house 1 = 48 m, i.d. = 0.5 mm)/Carbowax 20M (coated in house, 1 = 14 m, i.d. = 0.5 mm), type II Chirasil–Val[10] (coated in house, 1 = 50 m, i.d. = 0.5 mm) and Chirasil–Val (Applied Science, State College, PA16801, U.S.A., 1 = 25 m, i.d. = 0.3 mm)/Carbowax 20M (coated in house, 1 = 30 m, i.d. = 0.3 mm). The fused silica columns with chemically bonded phase were Carbowax 57 CB (Chrompack, The Netherlands, 1 = 50 m, i.d. = 0.32 mm) and CPSil 8 CB (Chrompack, The Netherlands, 1 = 51 m, i.d. = 0.32 mm, film thickness 1.3 μm). The Chirasil–Val and Chirasil–Val/Carbowax columns were operated at 80°C,[10,16] whereas for chromatographic runs on Carbowax 57 CB or CPSil 8 CB the columns were heated from 87–104°C at a rate of 1°C/min or from 80–87°C at 50°C/min and from 87–140°C at 6.2°C/min, respectively. Carrier gas was helium (1–2 ml/min).

Determination of residual soman concentration after degradation in rat blood

Supernatants of blood or plasma samples mixed with a 3-fold or 1.5-fold volume, respectively, of formate buffer (25 mM, pH 3.75) and centrifuged (20,000 g, 10 min), were assayed both enzymatically and gas chromatographically. In the enzymatic assay the supernatants were incubated with AChE for a fixed time (5 min). From the percentage of residual enzyme activity the soman concentration was evaluated with the aid of a calibration curve made with C(±)P(±)-soman.[17] In the GC-assay the supernatants were passed through a XAD-2 column which was subsequently eluted with ethyl acetate. After concentration at reduced pressure (7–8 kPa) to approximately

0.1 ml, the eluate was analyzed on a glass capillary SE-30 column (l = 35 m, i.d. = 0.7 mm), operated at 130°C with helium (5 ml/min) as carrier gas. The soman analogue $(CH_3)_2CHC(H)CH_3O(CH_3)P(O)F$ was used as an internal standard.

Enzyme assays

AChE was assayed titrimetrically (pH 7.5, 25°C) with acetylcholine perchlorate as a substrate. Carboxylesterase activities were determined both titrimetrically (pH 7.5, 25°C) and spectrophotometrically (pH 7.0, 25°C) with methyl butyrate (0.06 M) and *o*-nitrophenyl acetate (0.67 mM), respectively, as substrates.[17]

Work-up procedure for GC determination of the soman stereoisomers

Blood samples were mixed with a threefold volume of a 0.2 M acetate buffer (pH 3.5) containing 0.82 g saponine/1, 1.65 mM aluminum sulphate and a 100-fold excess of $(CH_3)_3CCH_2O(CH_3)P(O)F$ relative to the expected amount of C(±)P(±)-soman. After addition of internal standard the mixture is pressed through a Sep-Pak C_{18} cartridge. The analytes were eluted with ethyl acetate, after which the eluate was concentrated at reduced pressure (7.3 kPa).

Animal experiments

Male Wistar (WAG/Rij) rats, weighing 180–200 g and bred in the Medical Biological Laboratory TNO under SPF conditions, were used. For *in vitro* experiments blood was taken by heart puncture under nembutal anesthesia and collected in a syringe containing heparin (1000 IE).

Blood used for the development of the work-up procedure was obtained from rats anesthesized with sodium barbital (200 mg/kg, ip) and sodium hexobarbital (175 mg/kg, ip) and treated with atropine sulphate (50 mg/kg, ip) 5 min before administration of 1 LD50 (82 μg/kg) or 6 LD50 of C(±)P(±)-soman intravenously in the dorsal penis vein. The rats were kept alive with artificial respiration. After 1 h blood was taken via a carotid cannula.

RESULTS AND DISCUSSION

Qualitative analysis of soman stereoisomers and applications

GC separation of the four stereoisomers of soman A GC separation of the enantiomers of C(±)P(±)-soman was first achieved on a commercial column coated with the chiral phase Chirasil–Val, a copolymeric organosiloxane bound to L-valine tert.-butylamide. At optimal conditions, C(±)P(±)-soman was resolved in three instead of four peaks. The elution patterns of C(+)P(±)-soman and C(−)P(±)-soman showed that the enantiomeric pairs of soman are separated but that the retention times of the C(+)P(±)-epimers are identical (Figure 2). Resolution of all four stereoisomers was

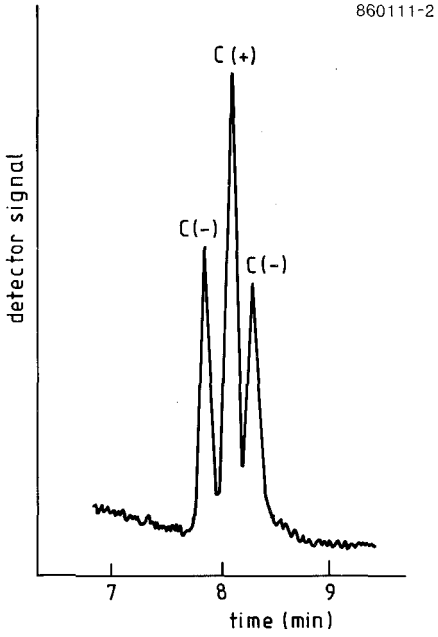


Figure 2 GC separation of the soman enantiomers on a commercial Chirasil–Val column at 80°C, precolumn pressure 103 kPa. The injection and FID-detector block of a Pye-104 gas chromatograph are held at 300°C. A 1-μl sample of 4 mM C(±)P(±)-soman solution in isopropanol is injected (split ratio 1:10). Reprinted with permission from *J. Am. Chem. Soc.* **103,** 4260 (1981). Copyright (1981) American Chemical Society.

obtained with a system consisting of the Chirasil–Val column coupled to a Carbowax 20M column,[16] which resolves[18] the epimers of C(±)P(±)-soman (Figure 3).

These results prompted us to synthesize Chirasil–Val and to coat glass capillary columns with the chiral phase obtained. The quality of the synthesized Chirasil–Val was found to vary between two resolution patterns. Columns coated with type I Chirasil–Val showed properties similar to the commercially available column and when combined with a Carbowax 20M column, separated the four stereoisomers as shown in Figure 3. On a column coated with type II Chirasil–Val the four stereoisomers are fully separated. Identification of the peaks of the chromatograms (see: *GC identification of anticholinesterase stereoisomers of soman*) indicates that the enantiomers are better separated on the type II Chirasil–Val column. The elution order of the enantiomeric pairs are peaks 1+2 and peaks 2+3 on type I Chirasil–Val, peaks 1+2 and peaks 3+4 on type I Chirasil–

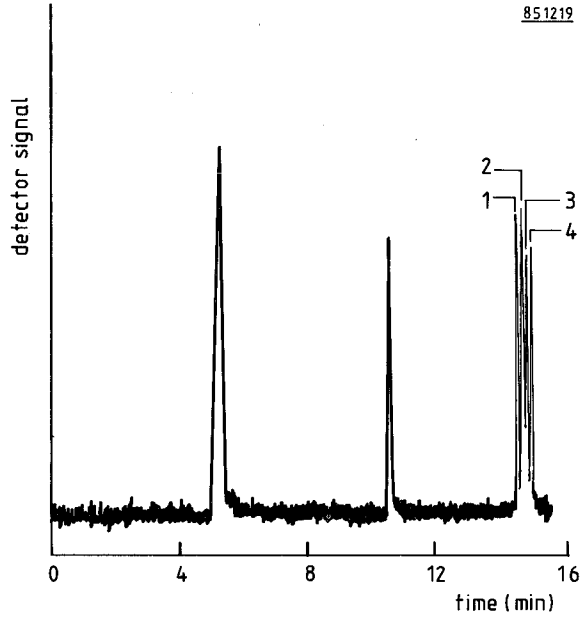


Figure 3 GC separation of the four stereoisomers of C(±)P(±)-soman on Chirasil–Val/Carbowax 20M. The Carbowax leg was connected with the injection port of the gas chromatograph. See Figure 2 for further data.

Val/Carbowax, whereas on type II Chirasil–Val the enantiomeric pairs are eluted as peaks 1+3 and peaks 2+4. It should be remarked that other investigations[19] also found variation in the chiral resolution quality of Chirasil–Val per synthesized batch.

GC identification of anticholinesterase stereoisomers of soman From kinetic analysis of the inhibition of bovine erythrocyte AChE with equimolar concentration of C(+)P(±)- or C(−)P(±)-soman Keijer and Wolring[4] showed that only one of the two stereoisomers of each preparation is a potent inhibitor of AChE. Accordingly, two peaks had almost disappeared in the elution pattern of the residual soman extracted from an incubation mixture of electric eel AChE (1.4 μM of active sites) with C(±)P(±)-soman. This stereospecific enzyme inhibition was applied to fully identify the peaks in the chromatograms. For that purpose experiments were carried out with chymotrypsin as an enzyme which can be used in a sufficiently high concentration (ca 1.8 mM of active sites) to determine the optical rotation of the residual soman after extraction. Upon incubation with a twofold molar excess of C(+)P(±)- as well as of C(−)P(±)-soman the residual soman had a significantly more positive optical rotation than before incubation.[16] GC analysis showed that chymotrypsin and AChE are preferentially inhibited by the same stereoisomers, now identified as the P(−)-epimers. So, the stereoisomers of soman corresponding to peak 1–4 of Figure 3 (Chirasil–Val/Carbowax) are the C(−)P(+)-, C(+)P(−)-, C(+)P(+)- and C(−)P(−)-isomers, respectively.

Stereospecific degradation of soman in rat blood GC analysis of the four stereoisomers was first applied to study the fate of C(±)P(±)-soman in rat blood and plasma *in vitro*. The concentration of each stereoisomer is the resultant of chemical hydrolysis, enzymatic hydrolysis by phosphorylphosphatase, irreversible binding to cholinesterases and other esterases and binding to other sites. After incubation for a relatively short time (10 min) only a small amount of the organophosphate is left as determined both enzymatically from its inhibitory effect on AChE and gas chromatographically on an achiral SE-30 column (Table I).

Soman concentrations were determined after adjusting the pH of the samples to 5.5 (0°C) to preclude further enzymatic degradation

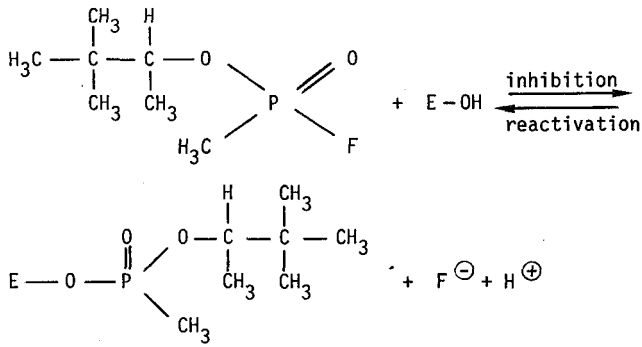
Table I Residual soman concentrations[a] in rat blood (A) and plasma (B) incubated with 1.4 and 2.3 μg C(±)P(±)-soman/ml for 10 min at 37°C, subsequently mixed with formate buffer (final pH 5.5) and centrifuged

Incubate	Treatment with NaF	Enzyme assay C(±)P(−)-soman (μg/ml)	GC assay total soman (μg/ml)	Chiral GC, peak ratios			
				C(+)P(+)	C(+)P(−)	C(−)P(+)	C(−)P(−)
A	none	0.095±0.009	0.082±0.016	–[b]	1[c]	–	1.3
	0.5 min	0.20±0.01	n.d.[d]		n.d.		
	30 min	0.21±0.02	0.42±0.03	0.8	1	0.8	0.9
B	none	0.19±0.02	0.18±0.04	–	1	–	1.3
	0.5 min	0.38±0.03	n.d.		n.d.		
	30 min	0.31±0.01	0.67±0.13	0.9	1	0.9	0.9

[a]Mean values and their standard deviations from three separate runs, calculated as concentrations in blood or plasma.
[b]Not detected.
[c]Arbitrarily set to 1.
[d]Not determined.

by phosphorylphosphatase.[7] In the enzymatic assay only the C(±)P(−)-epimers are determined because of the high reaction rate of these isomers with AChE in comparison with the C(±)P(+)-epimers. The correspondence between the data obtained in the enzymatic and the GC assays indicates that only C(±)P(−)-epimers are present in blood and plasma even after a short incubation.[17] This finding based on quantitative analysis was confirmed by qualitative GC assay on a Chirasil–Val/Carbowax column (Table I).

As a control experiment racemization was induced by incubation (30 min) with fluoride ions (2.5 mM). Indeed, the C(±)P(−)-soman concentration determined enzymatically becomes about half the total soman concentration as determined by achiral GC assay and four peaks with approximately the same height are observed in chiral GC analysis. Surprisingly, the treatment with fluoride ions at pH 5.5 highly increases the soman concentration. This increase is a fast process as appears from the considerable effect brought about within 0.5 min of treatment. Soman formed in this short period consists of C(±)P(−)-epimers as follows from chiral GC analysis after rapid extraction with ethyl acetate. Evidence is presented for parallel reactivation induced by fluoride ions of carboxylesterase which had been inhibited by soman. The percentage of reactivation after 0.5 min of fluoride treatment (pH 4.8–6.1) is found proportional to the C(±)P(−)-soman increase brought about under the same conditions. These results strongly indicate that added fluoride ions regenerate C(±)P(−)-soman by reversal of the inhibition reaction:



where E—OH represents the carboxylesterase.

From the relationship between percentage of reactivation and increase of soman concentration induced by fluoride ions at various conditions (pH, fluoride concentration) the carboxylesterase concentration in rat plasma was extrapolated as 2.6 μM corresponding closely to values reported in literature.[20,21] In this way an aspecific binding site contributing substantially to the fate of soman in rat blood was quantified.

The P(+)-epimers, with a low anticholinesterase activity, are much more rapidly degraded in rat blood than the P(−)-epimers which have a high anticholinesterase activity. Hence, it should be kept in mind that initial rates of overall degradation of soman as usually determined to characterize an enzyme reaction, are probably not relevant for the rate of detoxification of C(±)P(−)-soman in rat blood.[22,23]

Isolation of the four stereoisomers of soman The availability of a method for the separate analysis of the four stereoisomers of soman meets one of the requirements for their isolation as single isomers. The observed stereospecific enzymatic reactions offer the possibility for selective removal of stereoisomers. So, starting from C(+)P(±)- and C(−)P(±)-soman the two P(+)- and the two P(−)-isomers were isolated after incubation with chymotrypsin and in rabbit serum, respectively. At optimal conditions, the stereoisomers were obtained with more than 99% optical purity[9] on a 1 to 15 mg scale which is sufficient for toxicological investigations.

Quantitative analysis of soman stereoisomers and applications

Quantitative GC assay of the soman stereoisomers Quantitative GC assay of trace amounts of soman stereoisomers in biological material, in which these isomers may be highly labile, necessitates the use of internal standards whose properties are very similar to those of soman. We investigated the feasibility of two deuterated isomers of soman as isotopically labeled internal standards: D_3-C(±)P(±)-soman and D_{13}-C(±)P(±)-soman, in which the three hydrogen atoms of the P—CH_3 group and all hydrogen atoms of the pinacolyl moiety, respectively, are replaced by deuterium (Figure 4).

As the P(+)-epimers rapidly disappear in rat blood, separate

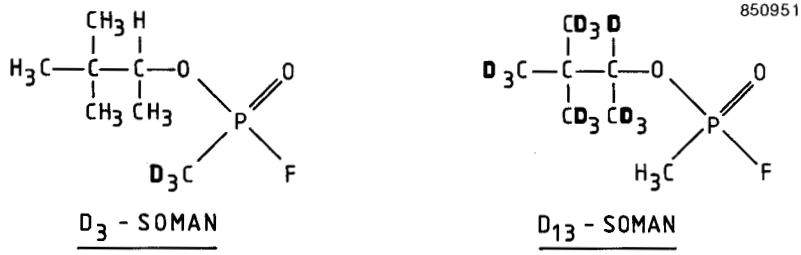


Figure 4 Chemical structures of partly deuterated soman analogues, for use as internal standards.

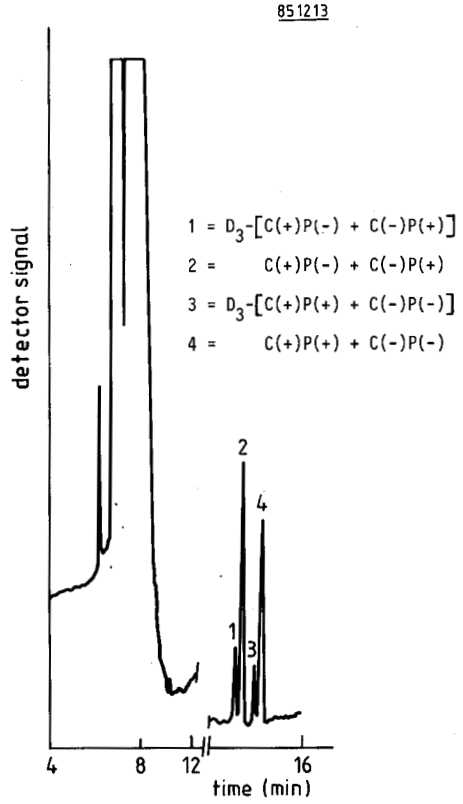


Figure 5 GC separation of the enantiomeric pairs of C(±)P(±)-soman and of D_3-C(±)P(±)-soman on a Carbowax 57 CB fused silica column. Cold on-column injection of a 3-μl sample in ethyl acetate with 10 s secondary cooling time. Alkali flame ionization detection. See Materials and Methods for further details.

assay of the residual P(−)-epimers can *a priori* be carried out on an achiral column, as already mentioned. We developed assays for these isomers on the basis of their resolution on a Carbowax column and on a SE-54 (CPSil 8) column. As shown in Figure 5 the enantiomeric pairs of soman and of D_3-soman are well separated on a Carbowax column, whereas on a SE-54 column the separate assay of the P(−)-epimers can well be performed by using D_{13}-soman as an internal standard (Figure 6).

The deuterated soman-isomers as such cannot be used as internal standards for the quantitative assay of all four stereoisomers of soman on type I or II Chirasil–Val columns, since only one or two of the deuterated stereoisomers are eluted separately from the soman stereoisomers. As D_3-C(−)P(+)-soman and the D_{13}-C(±)P(+)-

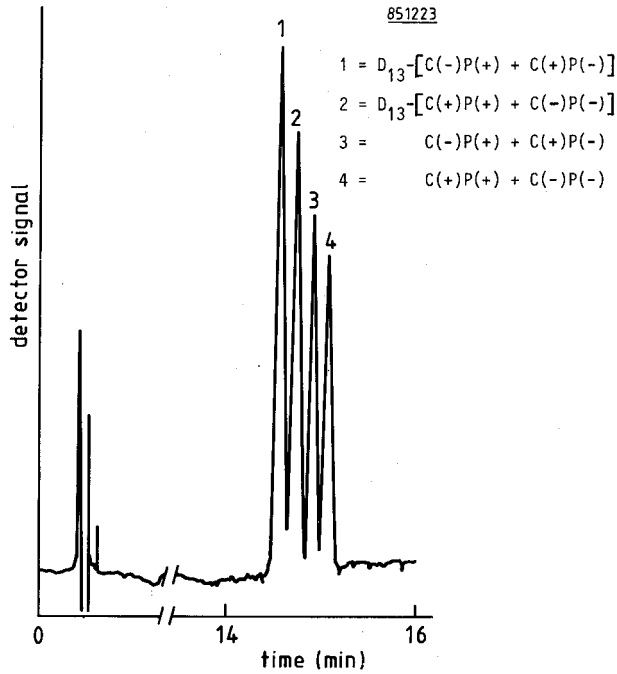


Figure 6 GC separation of the enantiomeric pairs of C(±)P(±)-soman and of D_{13}-C(±)P(±)-soman on a SE-54 (CPSil 8 CB) fused silica column. On-column injection of a 3-μl sample in ethyl acetate with 10s secondary cooling time. Alkali flame ionization detection. See Materials and Methods for further details.

epimers are fully resolved from the soman stereoisomers on a type I and a type II Chirasil–Val column, respectively, these isomers were isolated by analogy with the isolation of the non-deuterated soman stereoisomers, starting from D_3-C(−)P(±)-soman, prepared from (−)-pinacolyl alcohol, and from D_{13}-C(±)P(±)-soman, respectively. These preparations serve as convenient isotopically and stereochemically labeled internal standards for the quantitative assay of the four stereoisomers of soman (Figures 7 and 8).

Procedure for work-up of the four stereoisomers of soman from rat blood A quantitative determination of the stereoisomers of soman requires a work-up procedure during which the labile soman isomers are stabilized. On the basis of the work of Christen[7] we tried to block the activity of phosphorylphosphatase being especially active in hydrolysis of the P(+)-epimers, by acidification of the blood sample with a threefold volume of 0.2 M acetate buffer (pH 3.5). To check the stability of soman we used blood from anesthesized, atropinized and artificially respirated rats which had received 1 or 6 LD50 C(±)P(±)-soman 1 h earlier. In this way we obtained blood samples having their irreversible sites occupied as well as very low levels of residual C(±)P(−)-soman.

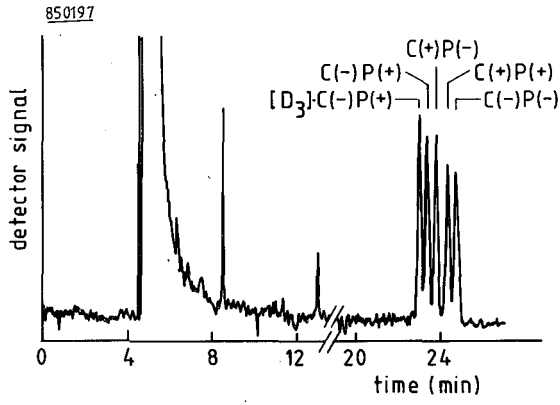


Figure 7 GC separation of the stereoisomers of C(±)P(±)-soman and of D_3-C(−)P(+)-soman on a type I Chirasil–Val/Carbowax 20M column. One side of the Carbowax column was connected with the injection port of the gas chromatograph. Injection of a 0.3-μl sample in isopropanol. Alkali flame ionization detection. See Materials and Methods for further details. Reprinted with permission from *Anal. Biochem.* **151,** 242 (1985).

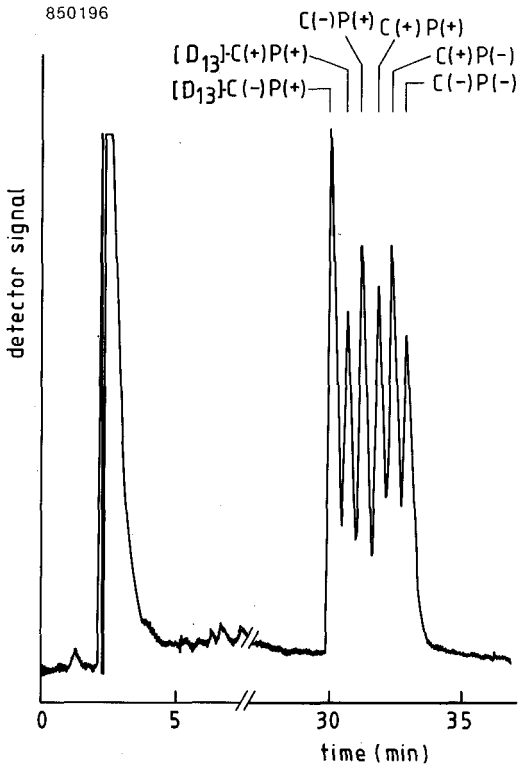


Figure 8 GC separation of the stereosiomers of C(±)P(±)-soman and of the D_{13}-C(±)P(+)-epimers on a type II Chirasil–Val column. Injection of a 0.3-μl sample in ethyl acetate. Alkali flame ionization detection. See Materials and Methods for further details. Reprinted with permission from *Anal. Biochem.* **151,** 242 (1985).

Incubation of C(±)P(±)-soman in pretreated blood acidified to a final pH of 4.2 provided an adequate stabilization of the P(+)-epimers for at least 40 min (Table II). However, the P(−)-epimers levels were too high after incubation for 5 min and increased considerably during further incubation for 40 min. Our above-mentioned results suggest that this increase of concentration may be due to carboxylesterase reactivation induced by fluoride ions, although present at low concentration (about 10 μM), at the acidic pH. Accordingly, the regeneration is completely suppressed after complexation of fluoride ions with aluminum ions (0.2 mM) (Table III). However,

Table II Effect of incubation (pH 4.2, 0°C) in presaturated[a] rat blood/0.2 M acetate buffer on the concentration of added C(±)P(±)-soman (10 ng/ml blood)

Isomer	Soman isomer[b] found as percentage of added amount after incubation for	
	5 min	45 min
C(+)P(+)	103±3	101±2
C(+)P(−)	304±35	1311±44
C(−)P(+)	98±1	92±6
C(−)P(−)	252±26	1141±73

[a]By pretreatment of anesthesized, atropinized and artificially respirated rats with 6 LD50 of C(±)P(±)-soman 1 h before sampling.

[b]Means±standard deviation ($n=3$) corrected for initial P(−)-values. P(+)-isomers are not detected in presaturated rat blood.

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Table III Stability of added C(±)P(±)-soman (10 ng/ml blood) in presaturated[a] rat blood/0.2 M acetate buffer (pH 4.2, 0°C) in the presence of 2.5 mM aluminum ions, without and with added neopentyl sarin

Isomer	Soman isomer[b] found as percentage of added amount			
	without neopentyl sarin after incubation for		with neopentyl sarin after incubation for	
	5 min	45 min	5 min	45 min
C(+)P(+)	98±10	106±4	92±0	93±0
C(+)P(−)	87±9	56±36	100±7	102±6
C(−)P(+)	99±6	95±3	94±3	98±6
C(−)P(−)	93±7	54±36	96±1	98±1

[a]By pretreatment of anesthesized, atropinized and artificially respirated rats with 1 LD50 of C(±)P(±)-soman 1 h before sampling.

[b]Means±standard deviation ($n=3$, without neopentyl sarin, or 2, with neopentyl sarin) corrected for initial P(−)-values. P(+)-isomers are not detected in presaturated rat blood.

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a new problem arises. At these conditions, the P(−)-epimers partly disappear in blood samples taken from rats which have been treated with 1 LD50. Apparently, irreversible binding sites had not completely been occupied by this pretreatment for 1 h. We, therefore, acidified the blood samples with acetate buffer containing the soman analogue neopentyl sarin $(CH_3)_3CCH_2O(CH_3)P(O)F$, in a 100-fold molar excess relative to the added amount of C(±)P(±)-soman, in addition to aluminum ions. From the results of these experiments, as given in Table III, it is concluded that in this blood–buffer mixture the four stereoisomers of soman are satisfactorily stabilized for further work-up.[10]

Extraction of the soman stereoisomers from the blood–buffer mixture to which an appropriate amount of internal standard was added, is performed by adsorption in a Sep-Pak C_{18} cartridge and subsequent elution with ethyl acetate. To increase sensitivity of the assay, eluates are concentrated at reduced pressure to a volume of about 0.1 ml. The overall recovery of the work-up procedure is about 50%[10] for each soman stereoisomer.

Minimum detectable concentration The described work-up procedure together with GC assay on a Chirasil–Val column (injection volume 0.3 μl) by using a NP-selective alkali flame detector allow the determination of soman concentrations down to ca 1 ng C(±)P(±)-soman/rat blood sample, i.e. ca 250 pg stereoisomer/rat blood sample (maximal 3 ml).

Samples obtained in toxicokinetic studies a relatively short time after injection of C(±)P(±)-soman, contain P(−)-epimers only which can be separately determined on a Carbowax or SE-54 column. By performing GC assay on a column with chemically bonded phase, larger sample volumes (up to 5 μl) can be analyzed with on-column or splitless (Grob) injection techniques. In this way the minimum detectable concentration of the P(−)-epimers is improved by one order of magnitude to 30 pg P(−)-isomer/rat blood sample.

Toxicokinetics of soman stereoisomers in the rat The described work-up and quantitative analysis method is now being used to follow blood levels of the stereoisomers of soman in the rat after C(±)P(±)-soman administration. In accordance with the *in vitro*

results the P(+)-epimers disappear rapidly from the blood. The concentration of the P(−)-epimers can be determined up to 4 h after injection of 6 LD50 of C(±)P(±)-soman.

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