similar to **1** were indeed detected by field desorption mass spectrometry.[1] In the solid state or solution, the repulsive electrostatic interactions should be reduced by interactions with anions (i.e., $CF_3SO_3^-$) or the solvent, increasing the stability of dications toward dissociation and allowing their isolation as relatively stable species.[1] Preliminary calculations indeed show that the hydrolysis of the neutral salt $(c\text{-}C_3H_2)_2O^{2+}\cdot 2Cl^-$ is much less exothermic than the hydrolysis of the dication.

In conclusion, the ether dications which were recently synthesized[1] are predicted to be essentially freely rotating and inverting around the central C–O–C bonds at room temperature. The preferred conformers range from bent planar (in **1**) to linear perpendicular in **2** and **3**.[18] Further studies on these and related[1] novel species (e.g., the cyclopropyldicarbinyl dication[19]) are under way and will be reported in a full paper.

Acknowledgment. We thank Professor P. J. Stang for bringing this problem to our attention, making his data available to us prior to publication, and valuable discussions. This research was partially supported by a grant from the United States—Israel Binational Science Foundation (BSF), Jerusalem, Israel.

Supplementary Material Available: Optimized structures of **1–4** (MINDO/3) and **1**, **3**, and **4** (STO-3G) in the form of *Z* matrixes (8 pages). Ordering information is given on any current masthead page.

(17) For example, the fragmentation of **1a** to $c\text{-}C_3H_2O^{+\cdot} + c\text{-}C_3H_2^{+\cdot}$ is endothermic by 14.1 kcal mol^{-1} (MINDO/3).

(18) We emphasize that our calculations apply to the isolated dications in the gas phase. Extrapolation of our conclusions to solution or the solid state, where interactions with the solvent and the anions are very important, should be done cautiously.

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(20) **Note Added in Proof**: The inclusion of correlation energy by using the perturbation procedure suggested by Møller and Plesset (see: J. A. Pople, J. S. Binkley, and R. Seeger, *Int. J. Quantum Chem.*, **S10**, 1 (1976)) has little effect on the calculated barriers to inversion at oxygen. Thus, compare the following inversion barriers (kcal mol^{-1}) at MP2/6-31G* and at RHF/6-31G* (in parentheses): H_2O 36.7 (35.6), $HOCH_2^+$ 24.2 (22.0), for **3a** → **3c** 22.4 (18.3). These results reinforce our conclusions in ref 12. We thank Dr. T. Clark (Erlangen) for running these MP2/6-31G* calculations.

Gas Chromatographic Separation and Identification of the Four Stereoisomers of 1,2,2-Trimethylpropyl Methylphosphonofluoridate (Soman). Stereospecificity of in Vitro "Detoxification" Reactions

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The nerve agent 1,2,2-trimethylpropyl methylphosphonofluoridate, $Me_3CCHMeO(Me)P(O)F$ (soman), is notorious for its extreme toxicity[1] and refractoriness with regard to the standard medical treatment for anticholinesterase intoxication.[2,3] Toxicological studies of this agent are complicated by the presence of two chiral centers in the molecule, leading to four stereoisomers.[4]

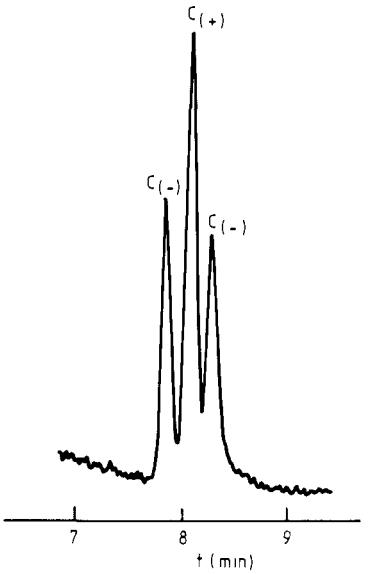

Figure 1. Gas chromatogram of soman after injection of a 1-μL sample of a 4-mM solution of soman in *i*-PrOH on a Chirasil-Val column (l = 25 m, ϕ = 0.3 mm; plate number 3×10^4, peak 1), column temperature 80 °C, precolumn pressure 103 kPa, injection split ratio 1:10. The injection block and the FID-detector block of the Pye-104 gas chromatograph were held at 300 °C.

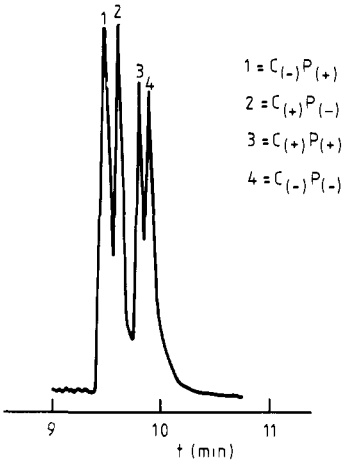

Figure 2. Gas chromatogram of soman on a Carbowax 20M/Chirasil-Val column. The Carbowax 20M column (l = 30 m, ϕ = 0.3 mm) was coupled directly to the same Chirasil-Val column as described in Figure 1. The Carbowax leg of the system was connected with the injection port of the gas chromatograph. The theoretical number of plates of the combined column is 1.3×10^5 (peak 1). See Figure 1 for further chromatographic conditions.

These isomers may vary widely in their (i) rate of inhibition of cholinesterases[7] and overall toxicity,[8] (ii) response to reactivators of the inhibited enzyme,[9] (iii) rate of aging of the inhibited enzyme,[7] and (iv) rate of "detoxification" in the body.[10,11] We now report a rapid and convenient GLC -method for the separation of the four stereoisomers of soman. These isomers have been identified and the usefulness of the method for toxicological studies is demonstrated.

In order to separate enantiometric compounds by means of GLC, an optically active stationary phase should be used.[12] We

(1) Warning: In view of its extreme toxicity, soman and other nerve agents should be handled only in specialized laboratories, where trained medical personnel is continuously present.

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(4) The absolute configuration of 3,3-dimethyl-2-butanol (pinacolyl alcohol) has been established[5] (the levorotatory enantiomer has the *R* configuration), whereas the absolute configuration around phosphorus in soman is, formally, still unknown.[6] We assign the four isomers of soman as $C_{(-)}P_{(-)}$, $C_{(-)}P_{(+)}$, $C_{(+)}P_{(-)}$, and $C_{(+)}P_{(+)}$, in which C stands for the pinacolyl moiety of soman.

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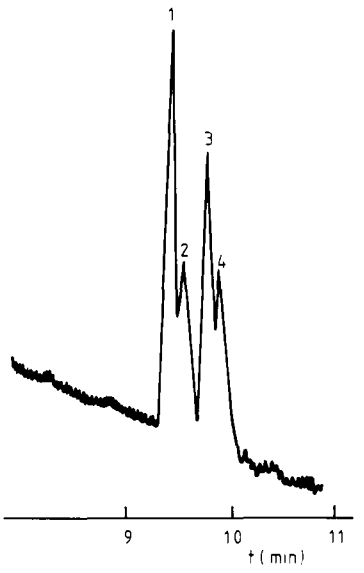

Figure 3. Stereoisomeric composition of residual soman, after incubation of electric eel AChE (ca. 1.4 μM of active sites) with a twofold molar excess of the inhibitor at pH 7.5, 25 °C, for 10 min.

have attempted such a separation of soman on a capillary column, coated with "Chirasil-Val". This phase is a copolymeric organosiloxane, bound to L-valine *tert*-butylamide.[13] Under optimal conditions (column temperature 80 °C), three instead of four clearly separated peaks could be observed (Figure 1). Using $C_{(-)}$-soman and $C_{(+)}$-soman, prepared from (*R*)(−)- and (*S*)-(+)-pinacolyl alcohol, respectively,[7] the two outer peaks in Figure 1 were identified as pertaining to the $C_{(-)}$ epimers of soman, whereas the large middle peak pertains to the $C_{(+)}$ epimers. Evidently, the enantiomeric pairs of soman are separated on the Chirasil-Val column, but the retention times of the $C_{(+)}P_{(+)}$ and $C_{(+)}P_{(-)}$ epimers are identical. Since it had been found previously in our laboratory that various stationary phases, e.g., Carbowax 20M, separate the epimers of soman,[14] the Chirasil-Val column was coupled to a capillary column, coated with Carbowax 20M. With this system, a satisfactory separation of the four stereoisomers of soman is obtained (Figure 2). By chromatography of the above-mentioned pairs of epimers $C_{(-)}$-soman and $C_{(+)}$-soman, the outermost peaks 1 and 4 in Figure 2 were identified as the two $C_{(-)}$ epimers, whereas peaks 2 and 3 pertain to the $C_{(+)}$ epimers.

A further identification of the four peaks was obtained by means of inhibition experiments with the enzymes acetylcholinesterase (EC 3.1.1.7, AChE; Sigma type V-S, isolated from electric eel) and bovine pancreas chymotrypsin (EC 3.4.21.1; Sigma type II). An aqueous solution of AChE (ca. 1.4 μM of active sites) was inhibited at pH 7.5, 25 °C, with a twofold molar excess of soman for 10 min. The residual soman was extracted with chloroform, concentrated, and analyzed. As shown in Figure 3, the enzyme was preferentially inhibited with the stereoisomers of soman corresponding to peaks 2 and 4 in the chromatogram. This result is in accordance with previous work from our laboratory,[7] carried out with bovine erythrocyte AChE, which showed that each of the epimeric pairs of $C_{(-)}$- and $C_{(+)}$-soman contains one stereoisomer with a high anti-AChE activity ($k_{inh} \geq 4.7 \times 10^5$ M^{-1} s^{-1}) and one isomer with a low activity ($k_{inh} \leq 10^2$ M^{-1} s^{-1}). The asymmetry in the pinacolyl moiety of soman has little influence on the anti-AChE activity.

Similarly, the stereospecific inhibition[15] of a concentrated aqueous solution of chymotrypsin (ca. 1.8 mM of active sites) with

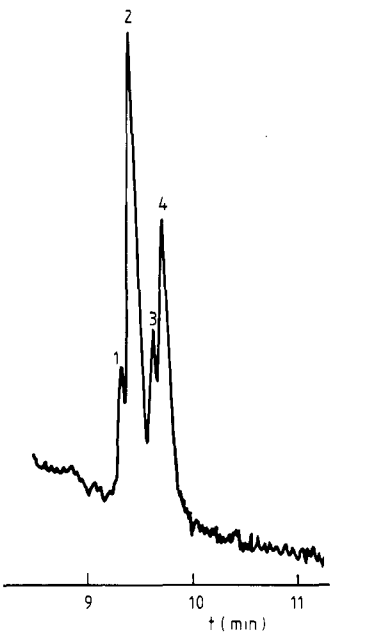

Figure 4. Stereoisomeric composition of soman, after incubation of soman (7.7 μM) in rat blood at 37 °C for 10 min. Qualitatively, a similar stereospecificity is observed upon partial breakdown of soman in rat liver homogenates and upon passage through excised guinea pig skin.

a twofold molar excess of $C_{(+)}$-soman [$[\alpha]^{20}_{578}$ +10.8 ± 0.4° (*c* 0.251, $CHCl_3$)] and $C_{(-)}$-soman [$[\alpha]^{20}_{578}$ −10.9 ± 0.4° (*c* 0.113, $CHCl_3$)] at pH 7.5, 25 °C, leads to the enrichment of residual soman with the isomers 1 and 3, respectively. In this case the optical rotations of the residual soman could be measured, after extraction of the enzyme solution with chloroform, i.e., $[\alpha]^{20}_{578}$ +30 ± 10° (*c* 0.019, $CHCl_3$) for residual $C_{(+)}$-soman and $[\alpha]^{20}_{578}$ −0.1 ± 0.1° (*c* 0.195, $CHCl_3$) for the residual $C_{(-)}$-soman.[16] Evidently, the enzymes AChE and chymotrypsin are preferently inhibited by the $P_{(-)}$ epimers of soman.[17] It follows that the stereoisomers of soman corresponding with peaks 1–4 (cf. Figure 2) are the $C_{(-)}P_{(+)}$, $C_{(+)}P_{(-)}$, $C_{(+)}P_{(+)}$, and $C_{(-)}P_{(-)}$ isomers of soman, respectively.[18]

The usefulness of our chromatographic system for toxicological studies is demonstrated by preliminary results of our current investigations on the fate of soman (i) in rat blood, (ii) in liver homogenates of rats, and (iii) on passage through skin excised from guinea pigs.

It has been demonstrated, e.g., by Christen et al.[10,11] that the nerve agent isopropyl methylphosphonofluoridate (sarin) is hydrolyzed in rat blood in a stereospecific way by a phosphorylphosphatase, which has been named "sarinase". We have now analyzed residual soman from rat blood, which had been incubated with soman (7.7 μM) at 37 °C for 10 min. As shown in Figure 4, the sarinase has preferentially hydrolyzed the $C_{(-)}P_{(+)}$ and $C_{(+)}P_{(+)}$ isomers of soman. It appears that the stereospecificity of the hydrolysis of soman in rat blood is analogous to that of sarin, the $P_{(+)}$ isomers being more rapidly hydrolyzed than $P_{(-)}$ isomers.[11,19]

Incubation of rat liver homogenates with soman (1–0.01 mM) at pH 7.5, 37 °C, for 10 min and subsequent analysis showed that, also in this case, the residual soman is highly enriched with the

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(16) The optical purity of residual $C_{(+)}$-soman is virtually 100%, while that of residual $C_{(-)}$-soman is 57%, as determined by GLC on the Carbowax 20M / Chirasil-Val column.
(17) See ref 6 for the stereospecific inhibition of AChE with the $P_{(-)}$ enantiomers of related chiral organophosphates.
(18) The first of the two peaks eluting from the single Carbowax 20M column upon injection of soman contains the $C_{(-)}P_{(+)}$ and $C_{(+)}P_{(-)}$ enantiomers, whereas the first-eluting peak on the single Chirasil-Val column (cf. Figure 1) corresponds with the $C_{(-)}P_{(+)}$ enantiomer of soman.
(19) For a similar stereospecific hydrolysis of soman by a "DFP-ase" from squid nerve and hepatopancreas, see: Gay, D. D.; Hoskin, F. C. G. *Biochem. Pharmacol.* **1979**, *28*, 1259. See also: Dudman, N. P. B.; Zerner, B. *J. Am. Chem. Soc.* **1973**, *95*, 3019.

$C_{(-)}P_{(-)}$ and $C_{(+)}P_{(-)}$ isomers. Finally, a similar stereospecific breakdown was observed on passage of soman (neat) through isolated guinea pig skin at ca. 30 °C. Initially ($t \leq 1$ h), only the $C_{(-)}P_{(-)}$ and $C_{(+)}P_{(-)}$ isomers of soman reappeared upon passage through the skin preparation.

Hence, in rat blood and liver homogenates of rats, as well as in guinea pig skin, the $P_{(+)}$ isomers of soman, which have a low anti-AChE activity, are "detoxified" more rapidly than the highly active $P_{(-)}$ isomers. It is tempting to speculate that in all these systems the same type of enzyme, i.e., a phosphorylphosphatase,[20,21] is responsible for the observed stereospecificity in the breakdown of the stereoisomers of soman.

Acknowledgment. We are indebted to Dr. C. van Hooidonk and B. I. Ceulen for their performance of the skin penetration experiments and C. van Dijk for her assistance in the enzymological experiments.

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Heats of Formation of *tert*-Butyl and Ethyl Radicals[1]

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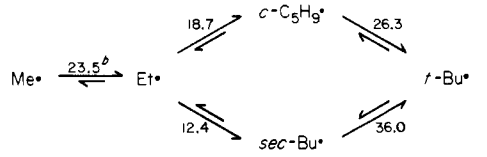
The C-H bond dissociation energies (BDE) in hydrocarbons are seldom known to better than ±1 kcal mol^{-1}. This is because they depend upon the heats of formation of alkyl radicals, $\Delta H_f(R\cdot)$, which are generally difficult to measure (eq 1). For example,

$$BDE(R-H) = \Delta H_f(R\cdot) + \Delta H_f(H\cdot) - \Delta H_f(R-H) \quad (1)$$

Scheme I. Equilibrium Constants Measured at 60 °C for Reaction 5[a]



[a] Error ±10%. [b] Measured at 0 °C.

previously reported values[2-7] of $\Delta H_{f,300}(t\text{-Bu}\cdot)$ cover the range 6.7-12.9 kcal mol^{-1} and give BDE for the simplest tertiary C-H bond, i.e., that in isobutane, as somewhere between 91.2 and 97.4 kcal mol^{-1}.[8]

In response to these problems, we have developed a system for measuring the *relative* heats of formation of alkyl radicals, which makes use of a "radical buffer".[9] With this technique, heats of formation of *tert*-butyl and ethyl radicals were measured relative to that of methyl, which serves as a reasonable standard,[10] $\Delta H_f(Me\cdot) = 34.4 \pm 0.7$ kcal mol^{-1}.

Our approach is based upon measurements of equilibrium constants, K, for the rapid exchange reactions which take place between alkyl radicals and alkyl iodides[11] (eq 5). Given the entropies of all of the components and the heats of formation of the iodides, a measurement of K leads to a value of $\Delta H_f(R\cdot) - \Delta H_f(R'\cdot)$.

The equilibrium constants, K, were measured by using EPR spectroscopy. Typically, an isooctane solution containing 0.5 M di-*tert*-butyl hyponitrite, 0.5 M triphenylarsine or triphenylboron, and 0.1-2.0 M of the two alkyl iodides was heated at 60 °C in the spectrometer cavity. The reaction scheme is described in eq 2-8. Relative radical concentrations were measured by double integration of appropriate lines in the EPR spectra.

$$t\text{-BuON=NOBu-}t \xrightarrow{\Delta} 2t\text{-BuO}\cdot + N_2 \quad (2)$$

$$t\text{-BuO}\cdot + MPh_3 \rightarrow t\text{-BuOMPh}_2 + Ph\cdot \quad (3)$$

$$Ph\cdot + RI/R'I \rightarrow PhI + R\cdot/R'\cdot \quad (4)$$

$$R\cdot + R'I \underset{}{\overset{K}{\rightleftharpoons}} RI + R'\cdot \quad (5)$$

$$R\cdot + R\cdot \rightarrow \text{nonradical products} \quad (6)$$

$$R\cdot + R'\cdot \rightarrow \text{nonradical products} \quad (7)$$

$$R'\cdot + R'\cdot \rightarrow \text{nonradical products} \quad (8)$$

From the point of view of these experiments, the reaction of phenyl radical with alkyl iodides is essentially irreversible.[12] The exchange reactions between alkyl radicals and alkyl iodides proceed with rate constants $>10^5$ M^{-1} s^{-1},[11-13] and hence the rates of these reactions are extremely rapid compared with the rates of loss of alkyl radicals by self-reaction, eq 6-8.[9] Therefore, the observed relative concentrations of the radicals are dependent upon the equilibrium constant[14] K. Moreover, values of K were independent of both the relative and absolute concentrations of the two iodides when these were varied by a factor of 10.

As an additional safeguard, the equilibria were, in some instances, established by using a second system for radical gener-

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