aqueous or organic phase may become saturated with the drug and can produce large errors in the log P measurements. Also, very lipophilic drugs may form micelles or microemulsions which would also produce errors with very high or very low lipophilicities that can be measured very easily, and because the quantitities of the drug used are extremely small, micelles or microemulsions present no problems.

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References and Notes

(1) J. M. McCall, *J. Med. Chem.*, **18**, 549 (1975).
(2) M. S. Mirrlees, S. J. Moulton, C. T. Murphy, and P. J. Taylor, *J. Med. Chem.*, **19**, 615 (1976).
(3) J. K. Miyake and H. Terada, *J. Chromatogr.*, **157**, 386 (1978).
(4) S. H. Unger, J. R. Cook, and J. S. Hollenberg, *J. Pharm. Sci.*, **67**, 1364 (1978).
(5) I. T. Harrison, W. Kurz, I. J. Massey, and S. H. Unger, *J. Med. Chem.*, **21**, 588 (1978).
(6) D. Henry, J. H. Block, J. L. Anderson, and G. R. Carlson, *J. Med. Chem.*, **19**, 619 (1976).
(7) J. K. Baker and C. Y. Ma, *J. Chromatogr.*, **169**, 107 (1979).
(8) R. F. Borne, R. L. Peden, I. W. Waters, M. Weiner, R. Jorden, and E. A. Coats, *J. Pharm. Sci.*, **63**, 615 (1974).
(9) D. O. Rauls and J. K. Baker, *J. Med. Chem.*, **22**, 81 (1979).
(10) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1967).
(11) G. H. Clowes, A. K. Keltch, and M. C. Krahl, *J. Pharmacol. Exp. Ther.*, **68**, 312 (1940).
(12) H. A. Chonle and A. Moment, *J. Am. Chem. Soc.*, **45**, 243 (1923).
(13) E. H. Volwiler, *J. Am. Chem. Soc.*, **47**, 2236 (1925).
(14) F. F. Blicke and R. H. Cox, "Medicinal Chemistry", Vol. IV, Wiley, New York, 1959, p 164–167.
(15) D. L. Taber and E. H. Volwiler, *J. Am. Chem. Soc.*, **56**, 1139 (1934).
(16) W. R. Gibson, E. E. Swanson, and W. J. Doran, *Proc. Soc. Exp. Biol. Med.*, **89**, 292 (1977).
(17) M. S. Tute, *Adv. Drug Res.*, **6**, 1 (1971).
(18) D. Hellenbrecht, K. F. Muller, and H. Grobecker, *Eur. J. Pharmacol.*, **29**, 223 (1974).

Antidotes to Organophosphate Poisoning. 2.[1] Thiadiazole-5-carboxaldoximes

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Three new nonquaternary oximes have been evaluated with respect to their antidotal activities against organophosphate poisoning. The oximes 1,2,3-thiadiazole-5-carboxaldoxime (**1**, pK_a = 7.6), 2-methyl-1,3,4-thiadiazole-5-carboxaldoxime (**2**, pK_a = 8.4), and 3-methyl-1,2,4-thiadiazole-5-carboxaldoxime (**3**, pK_a = 7.0) were prepared in yields of 56, 24, and 58%, respectively, by nitrosation of the corresponding methyl derivatives with isoamyl nitrite in the presence of potassium ethoxide. The new compounds are substantially more lipophilic and much less toxic than the well-known antidote P2S. They reactivate phosphylated AChE slowly. In the five animal species investigated, they disappear from the blood stream after iv administration at approximately the same rates as Obidoxime. It was shown that the disappearance of **1** from rabbit blood is not due to rapid renal excretion of the unchanged oxime, as is the case for Obidoxime and P2S. Since an unidentified derivative of **1** has been found in the urine, rapid metabolic conversion of **1** is most likely responsible. Therapeutic administration (ip) of **2**–**3** at 250 mg/kg, in combination with atropine, saves mice from four to five times the LD_{50} of sarin. Much lower doses (33–75 mg/kg) of **1** save rats and mice from five times the LD_{50} of sarin, whereas P2S cannot save mice from the same challenge dose. In contrast herewith, **1** does not save rats from five times the LD_{50} of paraoxon, whereas P2S is effective in this case. For oral prophylaxis against sarin, **1** is inferior to P2S. This inferiority may be partly ascribed to the combined effects of rapid absorption of **1** from the gastrointestinal tract and rapid elimination.

The oximes 2-[(hydroxyimino)methyl]-1-methylpyridinium methanesulfonate (P2S) and 1,1′-[oxybis(methylene)]bis[4-[(hydroxyimino)methyl]pyridinium dichloride (Obidoxime) are valuable antidotes in organophosphate poisoning.[2,3] Their therapeutic usefulness depends primarily on their abilities to reactivate the phosphylated[4] enzyme acetylcholinesterase (AChE), thereby restoring the normal transmission of nerve impulses.

In a previous paper,[1] we reported the synthesis and therapeutic activity of quaternary [(hydroxyimino)methyl]-2-methylisothiazolium salts, which are isosteric with the aforementioned pyridinium compounds. Unexpectedly, we also found that the nonquaternary isothiazole-5-carboxaldoxime reactivates phosphylated AChE, in spite of its rather high pK_a value (8.6). Moreover, in combination with atropine this oxime saved rats given several times the LD_{50} of isopropyl methylphosphonofluoridate (sarin). These results encouraged us to investigate related thiadiazole-5-carboxaldoximes. The introduction of an extra nitrogen atom into the ring of isothiazole-5-carboxaldoxime was expected to lower the pK_a toward the range 7.5–8.0, which is considered to be optimal for reactivation of inhibited AChE at physiological pH.[5,6] The presence of two nitrogen atoms and one sulfur atom in a five-membered (unsaturated) ring can lead to four different thiadiazole molecules, three of which were synthetically accessible. One oxime derivative of each of the three isomeric ring systems was synthesized for pilot studies of their various (physico) chemical and biological properties.

Results and Discussion

Synthesis and pK_a Values. The new oximes 1,2,3-thiadiazole-5-carboxaldoxime (**1**) and 2-methyl-1,3,4-thiadiazole-5-carboxaldoxime (**2**) were prepared in yields of 56 and 24%, respectively, by the same method as used by Goerdeler and Hammen[7] for 3-methyl-1,2,4-thiadia-

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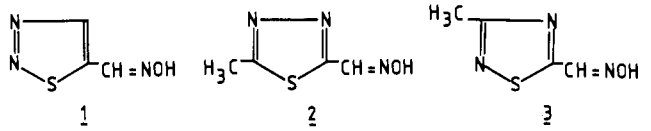
Table I. Thiadiazole-5-carboxaldoximes 1-3 Prepared by Nitrosation of the Appropriate Methyl Derivatives with Isoamyl Nitrite/Potassium Ethoxide



1 2 3

no.	yield, %	mp, °C	pK_a[a]	NMR absorption,[b] δ				formula[c]
				CH_3	CH (ar)	CH (al)	OH	
1	56	192	7.64		9.32	8.40	13.63	$C_3H_3N_3OS$
2	24	200	8.40	2.77		8.16	13.01	$C_4H_5N_3OS$
3	58	146	6.97	2.64		8.28	13.77	$C_4H_5N_3OS$

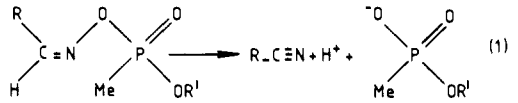
[a] The acid dissociation constants were determined potentiometrically in 0.1 M aqueous KCl, 25 °C. [b] The NMR spectra were measured in anhydrous Me_2SO-d_6 (Me_4Si). [c] Elemental analyses were correct within ±0.4% of the theoretical values for the elements indicated.

zole-5-carboxaldoxime (**3**), i.e., nitrosation of the appropriate methyl derivative with isoamyl nitrite in the presence of sodium ethoxide.

As indicated in Table I, the pK_a values of the new oximes are within the expected range. The 1,2,3-thiadiazole- and the 1,2,4-thiadiazole-5-carboxaldoximes in particular will be largely dissociated at physiological pH. The pK_a values indicate that, with regard to electron-withdrawing capacity, these two ring systems equal or surpass the pyridinium ring (e.g., P2S, pK_a = 7.8),[8] the α-keto group (e.g., hydroxyiminoacetone, MINA, pK_a = 8.3;[8] derivatives of oximinoacetic acid[9]), and the perfluoroalkyl moiety (e.g., trifluoroacetaldoxime, pK_a = 8.9).[10,11]

Nucleophilicity. Reactivation by oximes of phosphylated AChE is thought to proceed via a nucleophilic attack of the anion on the phosphorus atom. Therefore, the intrinsic reactivity of the oxime anion with regard to nucleophilic substitution at phosphyl centers is an important factor for the reactivation reaction, in addition to affinity for the inhibited AChE.[12] For an assessment of this reactivity, we determined the displacement of fluoride from sarin by **1-3**. In the displacement reaction, the phosphonylated oxime is formed with the release of fluoride ion and with the overall production of 1 equiv of acid. The acid production was measured by a pH-stat method (pH 7.6, 25 °C) when 1.0 mM sarin was incubated with 0.01 M of oxime. First-order reactions were observed, with rate constants of 1.19, 1.07, and 0.33 min^{-1} for **1**, **2**, and **3**, respectively. These rate constants are comparable to that of P2S under identical conditions (k_{obsd} = 0.35 min^{-1}).[1] As for **1** and **3**, the constants refer to reactions in which 1 equiv of acid is released per 1 mol of sarin. With **2**, however, 2 equiv of acid was liberated. Obviously, phosphonylated **2** is unstable, as is the case with many oximes (e.g., P2S), and further decomposition takes place, yielding a second proton. To verify that the acidimetrically determined constant corresponds with the rate of displacement of fluoride, we also studied the reaction of **2** with sarin with the help of a fluoride electrode. A constant of 0.96 min^{-1} was obtained, indicating that the initial reaction is, indeed, the rate-determining step.

The commonly encountered secondary reaction of phosphylated oximes is an elimination according to eq 1.



(1)

At first sight this reaction appears rather unlikely in the case of phosphonylated **2**, since **1** and **3** do not show this behavior even though they are more acidic than **2**.[13,6] This discrepancy might be explained, however, if phosphonylated **2** had the *Z* configuration which leads to a rapid trans elimination,[14,15] as shown in eq 1, in contrast with phosphonylated **1** and **3** which then should have the more stable *E* configuration.

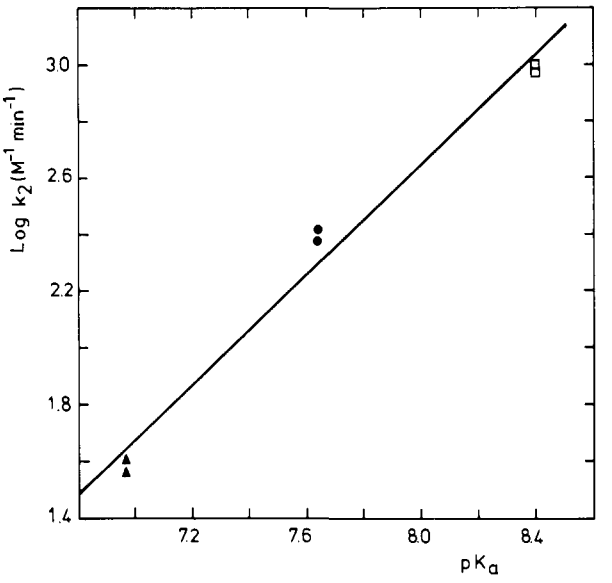


Figure 1. Log k_2 (M^{-1} min^{-1}) vs. pK_a for the reactions of **1** (●), **2** (□), and **3** (▲) with sarin in aqueous solution at 25 °C.

The second-order rate constants (k_2) for the reaction of the oxime anions with sarin were computed from data collected by the acidimetric procedure. When their logarithms were plotted vs. the pK_a values of the oximes, a good linear relationship was obtained (Figure 1), which can be described by eq 2. This Brönsted equation has a slope

$$\log k_2 = 0.97pK_a - 5.14 \quad (2)$$

$$r = 0.991$$

approaching unity, a high value characteristic for nucleophilic displacement at the phosphyl center;[16] so in this respect the new oximes **1-3** show the expected behavior.

Lipophilicity. The lipophilicity of drugs influences their pharmacokinetic behavior and therapeutic value; e.g., an increase in lipophilicity may improve the penetration into the central nervous system[17] and absorption from the intestines.[18] In view of a possible use of the new compounds as antidotes to organophosphates, the partition coefficients (*P*) of the oximes **1-3** for the system 1-octanol/aqueous phosphate buffer (pH 7.4, 25 °C) were determined as an index of their (apparent) lipophilicity. (At pH 7.4, the oximes are partially dissociated; so the measured values do not represent the true partition

Table II. Partition Coefficients (P) of Thiadiazole-5-carboxaldoximes 1-3 for the System 1-Octanol/Phosphate Buffer (pH 7.4, 25 °C)[a]

oxime	log P
1	+0.66
2	+0.74
3	+0.72
P2S	−3.24
Obidoxime	<−4
PAD	+1.41
MINA	+0.34

[a] Data for P2S, Obidoxime, PAD, and MINA are included for comparison.

Table III. Reactivating Potency and Affinity for AChE of the Thiadiazole-5-carboxaldoximes 1-3 and Some Reference Oximes

oxime	reactivat potency: % reactivat[a] after 2 h	6 h	24 h	affinity for AChE, % inhibn by 80 μM oxime[b]
1	5	19	32	<5
2	<5	10	16	<5
3	38	69	82	<5
MINA	25	56	74	<5
P2S	(45)[c]			12
Obidoxime	(86)[c]			13

[a] AChE inhibited with sarin was incubated with 1 mM oxime at pH 7.5 and 25 °C. The percentage restored AChE activity was assessed after 2, 6, and 24 h of incubation. [b] The affinity for AChE was determined by measuring the competitive inhibition of the enzyme activity in the presence of 3.2 mM acetylcholine, at pH 7.5 and 25 °C. [c] Reactivation after 3 min of incubation.

coefficients. Since the contribution of the anions to P is negligible, a correction for the extent of dissociation could have been applied. In view of the intended comparison of the properties in vivo, however, the use of an apparent P at a physiological pH was preferred.) As shown in Table II, the nonquaternary oximes **1-3** are substantially more lipophilic than the quaternary compounds P2S and Obidoxime but less than 2-[(hydroxyimino)methyl]-1-*n*-dodecylpyridinium iodide (PAD), which was designed by Wilson[19] for high lipophilicity.

Reactivating Potency. The oximes **1-3** were evaluated with respect to their ability to reactivate bovine erythrocyte AChE (acetylcholine hydrolase, EC 3.1.1.7) inhibited by sarin, by use of the method described previously.[1] Results are summarized in Table III, together with comparative data for the well-known reactivators MINA and P2S. Obviously, **1-3** reactivate phosphonylated AChE much more slowly than does P2S. Compounds **1** and **2** are even less active than MINA, whereas **3** is slightly more active than this oxime.

At higher concentrations of **1-3**, higher percentages of reactivation were attained, which indicates that the rather low extent of reactivation found at 1 mM is not caused by an enhanced rate of "aging" in the presence of the oxime. This was confirmed by kinetic analysis of the reactivation data as a function of the incubation time.

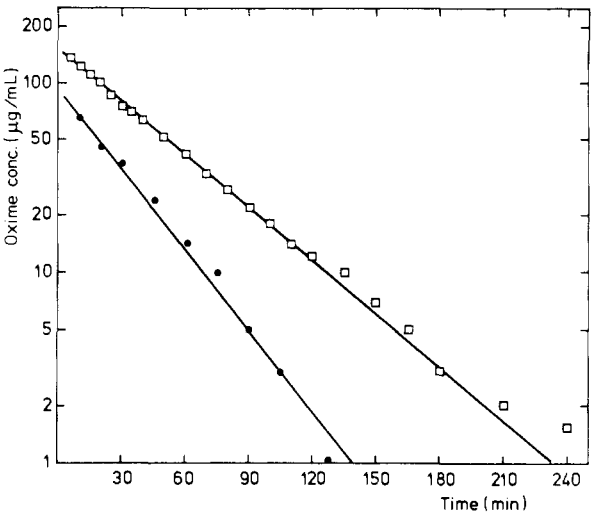


Figure 2. Oxime concentration in the blood of rabbits vs. time, after iv administration of 1 (●) and Obidoxime (□) at 50 mg/kg.

Since the aforementioned nucleophilicity of **1-3** toward sarin is not inferior to that of P2S, their relatively poor reactivating potency is probably due to a low affinity for the inhibited enzyme. This explanation is supported by the virtual absence of competitive inhibition of AChE by 80 μM solutions of **1-3**, whereas the same concentration of P2S or Obidoxime leads to an ca. 12% inhibition of the enzyme (Table III).

Biological Half-lives. Compounds **1-3** are sparingly soluble in water and were therefore converted into their sodium salts for animal experiments. The rates of disappearance of **1-3** from the blood of experimental animals after iv administration were determined in several species. For this purpose, blood samples were taken at intervals from the animals, erythrocytes and proteins were removed, and the UV absorbance in alkaline medium at the λ_{max} of the oxime anion was determined. In a few experiments, the spectrophotometrically determined oxime concentrations of blood samples were checked by means of high performance liquid chromatography (LC) after isolation of the oxime from plasma on an Amberlite XAD-2 column. In this way, both the identity and the amount of the compound determined by the UV method were verified. The high-performance LC results fully confirmed the reliability of the spectrophotometric method, since the differences remained within the experimental error (cf. Table IV).

A representative plot of the oxime concentration in rabbit blood vs. time, after an iv administration of **1** at 50 mg/kg, is given in Figure 2. The plot for Obidoxime is added for comparison.

Table IV. Spectrophotometrically Determined Biological Half-Lives of the Thiadiazole-5-carboxaldoximes 1-3 and of Obidoxime in the Blood of Various Animal Species

oxime[a]	biological half-life,[b] min in: mice	rats	guinea pigs	rabbit	goat
1	9.5 ± 1.2	19.9 ± 0.5[c]	20.9 ± 1.7	(26.2 ± 1.8)[d]	38.6 ± 2.5
2	10.4 ± 0.5	17.0 ± 1.3		27.7 ± 1.9	
3	7.0 ± 1.6	7.7 ± 0.8		13.9 ± 2.0	
Obidoxime	9.5 ± 0.5	15.4 ± 0.8	25.0 ± 1.4	32.3 ± 1.1	39.9 ± 2.9

[a] The sodium salts of the oximes were administered by iv injection at a dose of 50 mg/kg, except Obidoxime, of which 25 mg/kg was given. [b] With 95% fiducial limits. [c] A half-life of 19.6 ± 1.6 min was found by means of high-performance LC analysis. [d] A half-life of 24.4 ± 1.2 min was found after iv administration of 1 at 100 mg/kg.

Table V. Therapeutic Activity of the Thiadiazole-5-carboxaldoximes 1-3 and of P2S against Sarin in Mice[a]

treatment	LD_{50} (95% fiducial limits)[b] of sarin, μg/kg	therapeutic ratio
none	229 (221-242)	1.0
atropine alone	361 (304-444)	1.6
1 + atropine	1133 (1037-1290)	5.0
2 + atropine	967 (885-1041)	4.2
3 + atropine	1279 (1207-1357)	5.6
P2S + atropine	762 (683-823)	3.3

[a] The sodium salts of oximes 1-3 (250 mg/kg), P2S (47.2 mg/kg), and/or atropine sulfate (37.5 mg/kg) were administered ip, 1.5 min after sc administration of sarin. [b] Deaths were recorded after 24 h.

It is obvious from Figure 2 that the disappearance of 1 and of Obidoxime from the blood stream follows first-order kinetics. Consequently, the rates of disappearance can be expressed as half-lives. Similar results were obtained for 2 and 3 in rabbits, as well as for 1-3 and Obidoxime in mice and rats. In addition, the half-lives of 1 and Obidoxime were measured in goats and guinea pigs. In rabbits and goats, the decrease of the oxime concentration in blood with time could be followed by taking all blood samples from the same animal. In mice, rats, and guinea pigs, each blood level was obtained by averaging the results from four sacrificed animals. A summary of the results is given in Table IV.

It is known that the highly polar pyridinium oximes (e.g., P2S and Obidoxime) disappear from the circulating blood rather rapidly, due to fast renal excretion.[20] With the substitution of the pyridinium ring by the uncharged thiadiazole moiety, we expected an increased lipophilicity of the oximes. We hoped that this would diminish the rate of disappearance from the animal body and, consequently, extend their duration of action when used as antidotes. (For a discussion of the need for more persistent oximes, see ref 21.) This hope was not fulfilled, however, since 1-3 do not disappear from the blood of the experimental animals less rapidly than Obidoxime, in spite of the highly increased lipophilicity (cf. Table II).

Metabolism. In order to find out whether the rapid disappearance of 1 from the blood of experimental animals is due entirely to fast excretion or, alternatively, might involve a metabolic transformation, we collected urine for 24 h from rabbits given 1 at 125 mg/kg iv. Reverse-phase high-performance LC analysis (UV-detection at 280 nm) of this urine revealed that less than 0.2% of 1 was excreted unchanged into the urine. Moreover, on comparison of the chromatogram with that of urine from rabbits which had not been given 1, it appeared clearly that a new UV-absorbing product was present in the urine of rabbits which had been given 1. A small amount of this product was isolated by means of high-performance LC and an identification with the use of mass-spectrometry was attempted. Surprisingly, the mass spectrum (CI) appeared identical with that of oxime 1, although the new product was definitely distinct from 1 with regard to retention time in high-performance LC. In the mass spectrum, the peak of the parent ion was present (m/e 129), as well as the complete spectrum of the dehydration product of 1, i.e., 5-cyano-1,2,3-thiadiazole (M 111). As a possible conversion product of 1 with the same molecular weight (129), the corresponding 1,2,3-thiadiazole-5-carboxamide was studied. An authentic sample of this substance, however, was eluted from the high-performance LC column with a different retention time than the metabolite. As yet, the identity of the metabolite has not been established, but further attempts are in progress.[22]

The UV absorbance of the metabolite peak observed with high-performance LC suggests that a substantial fraction of 1 is excreted in the form of the metabolite with the urine. The conclusion appears warranted, therefore, that the rapid decrease of the blood levels of 1 is due to metabolism rather than to excretion of 1.

Therapeutic Activity. The new oximes could be safely administered in high doses, since they are of very low toxicity. Accurate LD_{50} determinations could not be performed because of the large quantities of oxime needed. For 1, the LD_{50} in mice (ip) was estimated at ca. 1250–1500 mg/kg, whereas doses of 2 and 3 of 1000 mg/kg caused no mortality. Likewise, no mortality was observed in rats which received a dose of 1 of 400 mg/kg ip.

When tested as therapeutic agents against sarin in mice, 2 and 3 appeared to be active only at a high dose level (≥167 mg/kg), in contrast with 1, which was active also at lower doses. The LD_{50} of sarin was determined in mice which were treated with 1-3 (250 mg/kg) and atropine. As shown in Table V, these high doses of 1-3 afford a significantly better antidotal effect against sarin than does P2S at the dose used (which was chosen within the most effective dose range: cf. Table VI).

Since 2 and 3 were effective only at impractically high dosages, 1 was selected for a further study of its antidotal activity. Various doses were tested against sarin in mice

Table VI. Therapeutic Activity of 1,2,3-Thiadiazole-5-carboxaldoxime (1) at Varying Doses, against Sarin in Mice and Rats and against Paraoxon in rats, in Comparison to That of P2S[a]

animal	organophosphate	challenge dose, μg/kg	oxime	survival:[b] dose of oxime, mg/kg						
				22	33	50	75	112	167	250
mouse	sarin[c]	1150 (5 × LD_{50})	1			1	6	7	8	9
mouse	sarin[c]	1150 (5 × LD_{50})	P2S			0	0	0	0	0
mouse	sarin[c]	690 (3 × LD_{50})	P2S		8	4	1	0	0	
rat	sarin[c]	775 (5 × LD_{50})	1	5	9	9	9	10		
rat	sarin[c]	775 (5 × LD_{50})	1			10	9	8	9	9
rat	sarin[c]	775 (5 × LD_{50})	P2S	9	10	9	8	9		
rat	paraoxon[d,e]	2685 (5 × LD_{50})	1	0	0	0	0	0		
rat	paraoxon[d,e]	2685 (5 × LD_{50})	P2S	8	8	7	7	1		

[a] Atropine sulfate (37.5 mg/kg) and either the sodium salt of 1 or P2S were administered ip, 1.5 min after sc administration of sarin. [b] Number of animals surviving for 24 h out of the 10 animals injected. [c] Treatment with atropine sulfate alone raises the LD_{50} of sarin in mice from 229 (221-242) to 361 (304-444) μg/kg and in rats from 155 (146-164) to 220 (197-239) μg/kg. [d] Since atropine sulfate affords an extremely high protection against paraoxon in rats, this antidote was omitted in these experiments. [e] In a separate experiment, the effect of the oximes on the time of onset of the symptoms was investigated in rats poisoned with five times the LD_{50} of paraoxon. Without treatment, the symptoms commenced after 2.9 ± 0.5 min; after treatment with P2S at 33 mg/kg, the onset time was 3.6 ± 0.3 min; after treatment with 1 at 100 mg/kg, an onset time 3.7 ± 0.5 min was observed.

Table VII. Effect of Pretreatment of Rats with Tri-o-cresyl Phosphate (TOCP) on the Therapeutic Activity of 1,2,3-Thiadiazole-5-carboxaldoxime (1) against Sarin[a]

pretreatment	therapy	ALD of sarin, μg/kg
none	atropine alone	460
none	atropine + 1	1500
TOCP	atropine alone	150
TOCP	atropine + 1	1000

[a] TOCP (50 mg/kg) was given sc, 24 h prior to sarin. The sc administration of sarin was followed after 1.5 min bij ip administration of the sodium salt of 1 (100 mg/kg) and/or atropine sulfate (37.5 mg/kg). The dose of sarin was varied in order to assess the approximate lethal dose (ALD).

and rats and against paraoxon (diethyl p-nitrophenyl phosphate) in rats (Table VI). At a dose of 75 mg/kg, 1 has a good therapeutic activity against five times the LD_{50} of sarin in mice, whereas P2S cannot save mice given this dose of sarin. Rats are saved from five times the LD_{50} of sarin by 1 at a dose of 33 mg/kg. Obviously, 1 is active against sarin at a relatively low dose. This result is remarkable when the poor reactivating potency of 1, relative to that of P2S, is taken into account (cf. Table III).

The effectivity of 1 as an antidote appears to vary with the animal species and to depend strongly on the type of anticholinesterase used, since (i) a much higher dose of 1 is needed in mice than in rats to obtain a comparable protection against sarin (>75 vs. 33 mg/kg) and (ii) 1 is very effective against sarin and superior to P2S, while it is unable to protect rats against five times the LD_{50} of paraoxon. Nor does 1 postpone significantly the onset of the symptoms of the intoxication (see footnote e to Table VI). On the other hand, P2S offers good protection against this dose of paraoxon, although also with this oxime no postponement of the onset of symptoms is observed (Table VI).

The apparent incongruity between the poor capacity of 1 to reactivate sarin-inhibited AChE in vitro and its outstanding therapeutic properties toward sarin intoxications in mice and rats is rather puzzling, unless the latter phenomenon is not caused by the restoration of AChE activity. Also, the contrast between the efficacy against sarin poisoning and the lack of effect toward five times the LD_{50} of paraoxon is rather striking. In an attempt to explain these seeming inconsistencies, we considered the possibility that the therapeutic action of 1 against sarin might be effected by the same mechanism which is responsible for the beneficial action of diacetyl monooxime (DAM) against sarin intoxications in the rat.[23] DAM proved to be a good reactivator of sarin-inhibited aliesterase, an enzyme which is rather abundant in rat blood.[24] The therapeutic effect of DAM appeared to be due to an enhancement of the decomposition of sarin in the blood stream via a cyclic process involving inhibition of this esterase by sarin, followed rapidly by reactivation by the oxime with the release of nontoxic hydrolyzed sarin.

To determine whether aliesterase might be involved in the results obtained with 1, we studied the effects of this oxime in rats which had been pretreated with tri-o-cresyl phosphate (TOCP) in order to inhibit this enzyme almost completely.[23] Next, the approximate lethal doses (ALD) of sarin were determined, 24 h later, with and without therapeutic treatment with 1 and/or atropine.

As shown in Table VII, the pretreatment with TOCP significantly increases the toxicity of sarin. The therapeutic activity of 1 is not offset by TOCP, however,

Table VIII. Prophylactic Activity against Sarin of 1,2,3-Thiadiazole-5-carboxaldoxime (1) and of P2S After Oral Administration to Rats[a]

dose of sarin,[b] μg/kg	oxime	survival:[c] time interval, min[d] 15	30	60
465 (3 × LD_{50})	1	8	4	0[e,f]
465 (3 × LD_{50})	P2S	10	9	10
620 (4 × LD_{50})	1	4	0[e]	0[e]
620 (4 × LD_{50})	P2S	9	8	9

[a] Both the sodium salt of 1 and P2S were administered at a dose of 100 mg/kg. [b] Atropine sulfate (37.5 mg/kg) was administered ip, 1.5 min after sc administration of sarin. [c] Number of rats surviving for 24 h out of the 10 rats injected. [d] Time interval between administration of oxime and injection of sarin. [e] Significantly different from P2S control ($p \leq 0.05$; two-tailed binomial test). [f] Significantly different from result at 15 min (two-tailed binomial test, $p \leq 0.05$).

Table IX. Blood Levels of 1,2,3-Thiadiazole-5-carboxaldoxime (1) and of P2S After Oral Administration (100 mg/kg) to Mice and Rats[a]

time after administn, min	blood levels, μg/mL: 1 rat	1 mouse	P2S rat	P2S mouse
15	22.9	31.1	1.6	3.2
30	22.1	23.6	3.9	4.8
60	8.4	6.9	4.1	4.5

[a] Each blood level is the mean of values from eight animals (SEM of 1, 1-2 μg/mL; SEM of P2S, <0.5 μg/mL).

demonstrating that the antidotal activity of 1 is not due to reactivation of phosphonylated aliesterase. The latter conclusion is confirmed by in vitro experiments: aliesterase from rat plasma was inhibited with sarin and was subsequently incubated with 0.5 mM DAM, P2S, and 1, respectively, at pH 7.2 and 37 °C for 60 min. With DAM, this resulted in the reactivation of 62% of the original aliesterase activity, whereas 1 and P2S reactivated only 28%.

Prophylactic Activity. In order to determine the prophylactic value of 1, this oxime was administered orally to rats 15, 30, or 60 min in advance of a lethal dose of sarin. Immediately after sarin, atropine was given for therapeutic treatment. For comparison, analogous experiments were done with equal doses of P2S.

The results in Table VIII show that P2S is quite effective against both doses of sarin (three and four times the LD_{50}), irrespective of the time interval between the administration of oxime and sarin. With 1, however, the time interval appears to be very important and, evidently, the prophylactic action is of short duration. Only at the shortest interval, against the lower dose of sarin, does the effect approach that of P2S, while after 60 min the prophylactic activity has disappeared completely.

The complete inefficacy of 1 when administered 60 min in advance is readily understood from the course of the blood levels of this oxime after oral administration (Table IX). As expected,[18] the nonquaternary and lipophilic oxime 1 is absorbed much more readily from the gastrointestinal tracts of rats and mice than P2S. The decreasing blood levels of 1 between 30 and 60 min after administration indicate that the absorption is virtually complete within 1 h. Thus, the combined effects of rapid absorption and rapid elimination ($t_{1/2}$ = 21 min; cf. Table IV) lead to such low blood levels of 1 at 60 min after administration that a protective effect of 1 against three

Table X. Reduction of the Hypothermia Induced in Rats by DFP or Paraoxon by the Thiadiazole-5-carboxaldoximes 1-3 and by Some Reference Oximes[a]

oxime	dose, mg/kg	% reduction of hypothermia	
		DFP	paraoxon
1	250	53	63
2	250	<10	<10
3	250	23	60
MINA	150	40	
OA-3	160	47	83
P2S	150	0	23
Obidoxime	150	60	
	80	40	30

[a] The reducing effect is expressed as a percentage of the hypothermia measured in control rats that received no oxime. One hour after the injection of organophosphate, the oxime was administered. The reduction in hypothermia was averaged over the 4th h after the injection of organophosphate. No meaning is to be attached to effects ≤10%.

to four times the LD_{50} of sarin is no longer observed.

Central Action. In order to assess their abilities to pass the blood–brain barrier, **1–3** were tested as antagonists of the hypothermia evoked in rats by the action of just sublethal doses of AChE inhibitors in the central nervous system.[25] In these experiments, rats were injected iv with either DFP (diisopropyl phosphorofluoridate) or paraoxon, and their body temperatures were followed for several hours. One hour after the injection, oxime was administered ip to the experimental animals, and the hypothermia in these rats was compared with that of the controls not receiving oxime. Reduction of the hypothermia indicates not only that the oxime reaches the central nervous system but also that it is able to restore the activity of functionally important AChE. The results were corrected for direct effects of the oxime on the hypothermia (not due to reactivation) by determinations of the influence on the body temperatures of rats which received soman (pinacolyl methylphosphonofluoridate) 1 h earlier. After 1 h, AChE inhibition caused by soman has become oxime resistent, and the effects of the oxime on body temperature cannot be ascribed to reactivation.[26]

Negligible therapeutic effects were obtained with **2**, as is shown in Table X, but **1** and **3** have a clear hypothermia-reducing activity, suggesting penetration of the blood–brain barrier and reactivation of inhibited brain AChE. For comparison, previously reported results[25] obtained with P2S, Obidoxime, and the centrally active oximes MINA and 3-(diethylamino)propyl oximinoacetate (OA-3) have been included in Table X.

Conclusions

By replacing the quaternary pyridinium ring of P2S with the nonquaternary thiadiazole ring systems, we obtained new antidotes for organophosphate poisoning which are substantially more lipophilic and less toxic than P2S and Obidoxime. Particularly interesting results were obtained with 1,2,3-thiadiazole-5-carboxaldoxime (**1**). Probably due to low affinity for AChE, **1** is a weak reactivator of phosphonylated AChE, although its pK_a value (7.6) for this reaction is almost optimal.[5,6] Nevertheless, therapeutic administration of **1**, together with atropine, saves rats and mice from five times the LD_{50} of sarin. In this respect, **1** equals or surpasses P2S. For oral prophylaxis against sarin, however, **1** is inferior to P2S. This fact may be partially explained by the combined effects of rapid absorption from the gastrointestinal tract and rapid elimination of **1**. Therapeutically, **1** is ineffective against five times the LD_{50} of paraoxon, in contrast with P2S.

We suggest that the therapeutic activity against sarin, the distinct central activity against DFP and paraoxon, as well as the rapid absorption of **1** from the gastrointestinal tract are partly due to its high lipophilicity. This allows **1** to attain therapeutically active concentrations at sites in the organism which are inaccessible to P2S. This difference in distribution may also play a role in the explanation of the different effects toward sarin and paraoxon, respectively. If this explanation is correct, the more favorable distribution evidently compensates for the low intrinsic reactivating potency of **1**, at least when used against sarin. A second favorable property of these new oximes is their relatively very low toxicity. In our view, these considerations warrant further investigations into the properties of uncharged, lipophilic oximes.

Because of their fairly lipophilic nature in comparison to the pyridinium oximes, we expected a more prolonged biological half-life for the thiadiazole oximes. The half-lives were not substantially different, however, from the value found for Obidoxime in the same species. Although somewhat disappointing, this result is not unprecedented, since the relationship between lipophilicity and biological half-life is far from straightforward: virtually all compounds with long half-lives are lipophilic but exceptions with regard to the inverse relationship are manifold.[27]

More surprising, perhaps, is the way of elimination. In the case of the pyridinium oximes Obidoxime and P2S, the short half-life is due to virtually complete renal excretion of the unchanged compound,[20,28] whereas the thiadiazole oximes (at least **1**) appear to disappear from the blood stream just as quickly because of a rapid metabolic conversion. This phenomenon may, in part, explain why these much more lipophilic oximes are not eliminated more slowly, but it also poses the as yet unanswered questions of the mechanism by which these oximes are converted and why the structurally related P2S is not similarly attacked.

Experimental Section

Melting points were determined on a Monoscop (Hans Bock, Frankfurt/Main, West Germany) and are uncorrected. NMR spectra were recorded on a JEOL C-60H spectrometer, using Me_2SO-d_6 as a solvent. IR spectra were obtained with a Grubb-Parsons GS-4 spectrometer. UV spectra of aqueous solutions were recorded by means of a Zeiss RPQ 20A spectrometer. Mass spectra were obtained with a VG micromass 7070F. Where results of analyses are indicated only by the symbols of the elements, the experimental values obtained for those elements were within ±0.4% of the theoretical values.

Sodium Salt of 1,2,3-Thiadiazole-5-carboxaldoxime (1). 5-Methyl-1,2,3-thiadiazole[29] (19.5 g, 0.195 mol) was nitrosated with isoamyl nitrite in the presence of potassium ethoxide in Et_2O/EtOH, according to the procedure of Goerdeler and Hammen.[7] The crude potassium salt of **1** (29.5 g) was dissolved in water and the solution was acidified with 2 N HCl to pH 4.0. Next, the precipitated product **1** was filtered off and recrystallized twice from H_2O/EtOH (3:1): yield 14.2 g (0.110 mol, 56%) of colorless **1**, mp 191–192 °C; UV (0.1 N NaOH) λ_{max} 318 nm (ϵ 7160); IR ($CHCl_3$) ν_{OH} 2675 cm^{-1}; ^1H NMR showed that the product was isomerically pure.

A 12.0-g sample (0.093 mol) of **1** in EtOH was treated at 0 °C with an equivalent amount of sodium ethoxide in EtOH. The solvent was evaporated and the residue was washed with Et_2O. Next, the sodium salt was dissolved in the minimal volume of Me_2CO/EtOH (1:3) and was subsequently precipitated with Et_2O. After filtration, the yellow product was dried over P_2O_5 at atmospheric pressure: yield 15.0 g (0.080 mol, 86%). Anal. ($C_3H_2N_3OS$·Na·$2H_2O$) C, H, N, S.

Sodium Salt of 2-Methyl-1,3,4-thiadiazole-5-carboxaldoxime (2). 2,5-Dimethyl-1,3,4-thiadiazole (55.0 g, 0.482 mol), prepared[30] from the condensation of diacetyl hydrazine with P_2S_5,

was nitrosated as described above for 1. This procedure gave 16.7 g (0.117 mol, 24%) of colorless 2, after recrystallization from MeOH/H_2O (1:2): mp 198–200 °C; UV (0.1 N NaOH) λ_{max} 298 nm (ϵ 9640); IR ($CHCl_3$) ν_{OH} 2750 cm^{-1}; ^1H NMR showed that the product was isomerically pure.

A 10.0-g sample of 2 (0.070 mol) was converted into its yellow sodium salt (10.9 g, 0.070 mol, 94%), as described for the sodium salt of 1. The product was dried over P_2O_5 at reduced pressure. Anal. ($C_4H_4N_3OS$·Na) C, H, N, S.

Sodium Salt of 3-Methyl-1,2,4-thiadiazole-5-carboxaldoxime (3). 3,5-Dimethyl-1,2,4-thiadiazole (20.0 g, 0.175 mol), obtained by oxidative ring closure of *N*-[(1-aminoethylidene)-thio]acetamide hydrochloride,[31] was nitrosated according to Goerdeler and Hammen.[7] The product was recrystallized from benzene: yield 14.5 g (0.101 mol, 58%; lit.[7] 56%) of colorless 3; mp 145–146 °C (lit.[7] mp 147 °C); UV (0.1 N NaOH) λ_{max} 305 nm (ϵ 8570); IR ($CHCl_3$) ν_{OH} 2715 cm^{-1}. According to ^1H NMR spectroscopy, 3 was isolated as a single isomer.

The yellow sodium salt of 3 (5.3 g, 0.032 mol, 92%) was obtained from 3 (5.0 g, 0.035 mol), as described above for 1. The hygroscopic product was dried over P_2O_5 at reduced pressure. Anal. ($C_4H_4N_3OS$·Na) C, H, N, S.

5-Cyano-1,2,3-thiadiazole (4). A 2.00-g sample (15.5 mmol) of 1,2,3-thiadiazole-5-carboxaldoxime (1) was refluxed with Ac_2O (5.00 g, 49.0 mmol) for 1.5 h. Concentration in vacuo of the reaction mixture left a residue, which was purified by repeated sublimations until a constant melting point (64.5–65.0 °C) was obtained: yield 1.55 g (13.9 mmol, 89.7%). The colorless product turns blue in the light and should be kept in the dark. The mass spectrum (EI) of 4 showed major peaks at m/e 111 (M^+), 83 (111 − N_2), 57 (83 − CN), 56 (83 − HCN), and 51 (83 − S). Anal. (C_3HN_3S) C, H, N, S.

1,2,3-Thiadiazole-5-carboxamide (5). A sample of 4 (1.10 g, 9.90 mmol) in 10.7 mL of 0.33 N NaOH in EtOH/H_2O (1:1.14, v/v) was refluxed for 7.5 h. Next, the reaction mixture was dried in vacuo and the residue was recrystallized from H_2O until a constant melting point (162–163.5 °C) was obtained: yield 0.91 g (71.2%) of colorless 5, which turns blue in the light; MS (CI) m/e 130 $(M + 1)^+$ 102, 101, 87, 59, 58, 45, 44. Anal. ($C_3H_3N_3OS$) C, H, N, S.

Reactions of 1–3 with Sarin. Rate Measurements. The acidimetric method for the determination of the reaction rates of 1–3 with sarin in aqueous solution was described in a previous paper.[1] The procedure in which a fluoride electrode is used has also been published.[32]

Partition Coefficients. For the determination of partition coefficients, a solution of the oximes was made in pH 7.4 sodium phosphate buffer (0.1 M), of which 1 volume was shaken with 10 volumes of 1-octanol for 1.5 h at 25 °C (usually the Na salt of the oxime was taken; prior to use, the buffer was extracted with 1-octanol; when necessary, the pH of the oxime solution was adjusted to 7.4). Next, the phases were separated by centrifugation, and the oxime content of the aqueous phase was determined spectrophotometrically and compared with the oxime concentration in the original solution. Each partition coefficient was determined twice in triplicate, at an oxime concentration of 2.5 mg/mL (12–15 mM); the averaged values (used for log P in Table II) had standard errors of ≤6%. Less extensive determinations were also performed at lower concentrations (1 and 0.1 mM) which showed P to be independent of oxime concentration in the range studied. Use of 1 instead of its Na salt yielded identical values for P.

Reactivation of Inhibited AChE. The reactivation experiments in vitro were performed as described previously,[1] except for the inhibition by sarin of bovine erythrocyte AChE (Winthrop Laboratories Inc.). The phosphonylated enzyme was prepared by incubation with 0.1 μM sarin in a 6.6 mM Veronal buffer (pH 9.0) for 100 min at 0 °C.

Reactivation of Inhibited Aliesterase from Rat Plasma. Nine parts of rat blood plasma were incubated with one part of 10 mM *p*-(hydroxymercuri)benzoate solution in 20 mM glycylglycine buffer for 1.5 h (pH 7.9, 37 °C) in order to inhibit the sarinase activity.[24] Next, the aliesterase activity was inhibited by incubation of the plasma with 10 μM sarin for 1 h at 37 °C. The inhibited enzyme was reactivated at pH 7.2 and 37 °C. To this end, 1 volume of the plasma treated in the above-mentioned manner was mixed with an equal volume of a 1 mM oxime solution in 50 mM Veronal buffer, pH 7.0. Samples for the assay of aliesterase activity were taken after 1 h of reactivation. The aliesterase activities were determined spectrophotometrically with *o*-nitrophenyl acetate as the substrate.[33] In this assay, an enzyme sample (20–40 μL) was added to 3 mL of 0.67 mM *o*-nitrophenyl acetate in 50 mM Veronal buffer, pH 7.0, and the increase in absorbance at 368 nm after 5 min of incubation at 37 °C was measured. Spontaneous hydrolysis of substrate can be neglected under these conditions. Control experiments with plasma which was not treated with sarin were performed simultaneously, with and without added oxime.

Biological Half-lives. Animals used for the determination of biological half-lives were: hybrid mice of the type CC 57 (CBA♂ × C57BL♀), inbred rats of the WAG (small Wistar) strain, outbred guinea pigs of an unnamed albino strain (maintained by CPB, Zeist), and outbred rabbits of the Flemish Giant breed. The oxime was injected intravenously in a tail vein (mice and rats), penis vein (guinea pig), ear vein (rabbits), or the extrajugular vein (goat). Oximes were given orally (100 mg/kg) by esophageal intubation to animals which had been fasted overnight.

Blood was collected after decapitation (mice and rats), by heart puncture (guinea pigs), from a vein of the other ear (rabbits), or from the extrajugular vein (goat). The heparinized samples were centrifuged immediately after collection and the plasma was removed for analysis.

A. Spectrophotometric Analysis. Aliquots of the plasma (0.2 mL) were deproteinized by the addition of 0.3 mL of 20% aqueous trichloroacetic acid and subsequent centrifugation for 15 min at 1500*g*. Then, 0.2 mL of 5 N aqueous NaOH was added to 0.35 mL of the supernatant, and the absorbance at λ_{max} (320 nm) of the oxime anion was measured. The oxime concentrations in the original samples were read from calibration curves, which were constructed from the results obtained with known quantities of oxime added to 1-mL blood samples which were carried through the same procedure.

B. High-Performance LC Analysis. The analyses of 1 were carried out at ambient temperature with a system consisting of two Waters Associates 6000A pumps, a solvent programmer (Waters Model 660), a Valco universal injector, steel columns (length 25 cm, internal diameter 4.6 mm) containing either silica for normal phase analysis (Lichrosorb Si-60-5; Merck) or C_{18}-modified silica (RP-18; Merck) for reverse-phase chromatography, packed according to the procedure of Linder c.s.,[34] and a variable-wavelength UV detector (Tracor, Model 970). Peak areas were measured with an Infotronics CRS 304 electronic integrator.

Since the plasma samples were analyzed on the normal phase column, the oxime had to be transferred to an organic phase. For this purpose, 1.0 mL of plasma was brought on an Amberlite XAD-2 column (length 8 cm, diameter 1.5 cm), on which low-molecular-weight organic compounds are absorbed from the aqueous phase.[35] The column was washed with 25 mL of aqueous HCl (pH 2.3) in order to remove salts, proteins, etc. Next, the resin was dried in a stream of air and then extracted with two 10-mL portions of EtOH in order to desorb the oxime. The combined extracts were concentrated in vacuo to a volume of 5 mL for high-performance LC analysis. In separate experiments it was shown that >90% of the oxime 1 in plasma is recovered from the XAD-2 column by means of this procedure.

The high-performance LC analyses on the normal phase column were performed with the eluent hexane/EtOH (5:1) to which ca. 0.1% of AcOH was added in order to obtain symmetric peaks. The oxime was eluted with a capacity factor $k' = 1.4$; it was detected by means of its UV absorption at λ_{max} 280 nm. The oxime concentrations in the original blood samples were read from calibration curves which were constructed from the results obtained with known quantities of oxime added to blood samples that were carried through the same procedure.

C. Statistical Evaluation. For the statistical evaluation of the half-lives in mice, rats, and guinea pigs, a computer program was used in which, first, the results for each time interval were averaged and then a weighting factor (number of data points divided by the variance) was assigned to each value. Finally, the half-lives and their 95% fiducial limits were computed. For rabbits and goats, the half-life for each animal was computed first and then the results were averaged.

Metabolism and Excretion. Urine (96 mL) was collected from a rabbit (weight 2.5 kg) maintained in a metabolism cage for 24 h following the iv injection of 1 (50 mg/kg). A piece of a small-mesh wire netting was placed within the collecting funnel in order to prevent the urine in the storage tank to become contaminated by feces.

Normal phase high-performance LC analysis of the oxime in urine by the same procedure as used for blood appeared to be inconvenient in view of the multitude of compounds in the urine eluted with similar retention times as the oxime. Therefore, reverse-phase high-performance LC was used for oxime analysis in urine. After adjustment of the original pH (8.1) of the urine to pH 3.0 with concentrated HCl, a sample was injected directly on the reverse-phase column. The eluent was H_2O/MeOH (4:1; 0.3%, v/v, AcOH added). The oxime was eluted with a capacity factor $k' = 2.8$ (detection by its UV absorption at λ_{max} 280 nm). The oxime concentration in the urine (2.0 μg/mL) was read from a calibration curve, which was constructed from results obtained with known quantities of oxime added to control urine. From this value the amount of 1 excreted unchanged with the urine was calculated.

Elution of the urine sample from the reverse-phase column with H_2O/MeOH/AcOH (90:10:0.6, v/v; UV detection at 280 nm) and comparison of the obtained chromatogram with that of control urine revealed one additional component ($k' = 2.7$) that was present only in the urine of rabbits which had been given 1. The eluate fractions containing this unknown component were collected and the solvent was evaporated in a stream of air. Mass spectrometry (CI) showed unequivocally that the unknown component is a derivative of 1, although the structure has not been elucidated so far (see note added in proof, ref 22).

Toxicological Procedures. Female CC57 mice weighing 18–22 g and female WAG rats weighing 150–170 g were used.

Sarin was administered subcutaneously in aqueous solution (mice, 10 mL/kg; rats, 2.5 mL/kg). Paraoxon was administered subcutaneously in propylene glycol to rats (0.25 mL/kg). Oximes and atropine sulfate were administered intraperitoneally in aqueous solution (mice, 10 mL/kg; rats, 1 mL/kg). Deaths were ascertained at 24 h and LD_{50} values were calculated by means of the probit method.

Central Action. Groups of at least eight rats, briefly anesthesized with hexobarbital, were injected into the jugular vein with DFP (1 mg/kg) or paraoxon (0.4 mg/kg). Their body temperatures were registered at hourly intervals for several hours. Oxime was injected ip after 1 h. The hypothermia in these animals was compared to that in control rats not receiving oxime. The reduction in hypothermia as reported in Table X is the average of the values measured after 3 and 4 h, respectively; it is given as the percentage of the control value. These percentages have been corrected for the (usually small) decrease in body temperature caused by some of the oximes due to effects other than reactivation of AChE. These corrections were obtained in control experiments in which rats were given soman (32 μg/kg). Due to rapid aging of soman-inhibited AChE, effects caused by oximes injected after 1 h cannot be ascribed to reactivation. To illustrate the procedure and to give an indication of the accuracy of the percentages in Table X, as a numerical example the calculations for oxime 1 and DFP are given: For each group of rats the individual hypothermia's after 3.5 h were calculated and, subsequently, the mean value ± SEM was computed. In the control group, DFP induced a hypothermia of 2.73 ± 0.08 °C ($n = 8$). In the experimental group treated with 1, the corresponding value was 1.87 ± 0.08 °C ($n = 8$) or 0.86 ± 0.11 °C less. Soman induced a hypothermia of 2.47 ± 0.15 °C ($n = 8$), which was increased by 1 to 3.05 ± 0.16 °C ($n = 8$), being 0.58 ± 0.22 °C more. Consequently, the total beneficial effect of 1 against DFP amounted to 0.86 + 0.58 = 1.44 ± 0.25 °C, which is 53 ± 9% of the hypothermia caused by DFP. For a more detailed description and an evaluation of this method, see ref 25 and 36.

References and Notes

(1) For Part 1, see H. P. Benschop, A. M. Van Oosten, D. H. J. M. Platenburg, and C. Van Hooidonk, *J. Med. Chem.*, **13**, 1208 (1970).
(2) F. R. Sidell, *Clin. Toxicol.*, **7**, 1 (1974).
(3) W. D. Erdman and M. V. Clarmann, *Dtsch. Med. Wochenschr.*, **88**, 2201 (1963).
(4) "Phosphylated" covers phosphorylated, phosphonylated, and phosphinylated; see R. F. Hudson and L. Keay, *J. Chem. Soc.*, 1859 (1960).
(5) Y. Ashani and S. Cohen, *J. Med. Chem.*, **14**, 621 (1971).
(6) I. Hagedorn, I. Stark, and H. P. Lorenz, *Angew. Chem.*, **84**, 354 (1972).
(7) J. Goerdeler and H. W. Hammen, *Chem. Ber.*, **97**, 1134 (1964).
(8) A. L. Green and B. Saville, *J. Chem. Soc.*, 3887 (1956).
(9) I. N. Somin and S. G. Kuznetsov, *Khim.-Farm. Zh.*, **2**, 39 (1968).
(10) B. L. Dyatkin, E. P. Mochalina, and I. L. Knunyants, *Tetrahedron*, **21**, 2991 (1965).
(11) R. L. Salvador, M. Saucier, D. Simon, and R. Goyer, *J. Med. Chem.*, **15**, 646 (1972).
(12) I. B. Wilson and H. C. Froede, "Drug Design", Vol. II, E. J. Ariens, Ed., Academic Press, New York, 1971, p 213.
(13) J. H. Blanch and J. Andersen, *J. Chem. Soc. B*, 169 (1968).
(14) J. H. Blanch and O. T. Onsager, *J. Chem. Soc.*, 3734 (1965).
(15) G. M. Steinberg and S. Soloman, *Biochemistry*, **5**, 3142 (1966).
(16) R. F. Hudson, "Structure and Mechanism in Organo-Phosphorus Chemistry", Academic Press, London, 1965, p 99.
(17) L. S. Shanker, *Antimicrob. Agents Chemother.*, **5**, 1044 (1965); N. Bodor, E. Shek, and T. Higuchi, *Science*, **190**, 155 (1975).
(18) E. J. Lien, in "Drug Design", Vol. V, E. J. Ariens, Ed., Academic Press, New York, 1975, p 84.
(19) I. B. Wilson, *Biochim. Biophys. Acta*, **27**, 196 (1958).
(20) F. R. Sidell and W. A. Groff, *J. Pharm. Sci.*, **59**, 793 (1970).
(21) H. P. Benschop, L. P. A. De Jong, J. A. J. Vink, H. Kienhuis, F. Berends, D. M. W. Elskamp, L. A. Kepner, E. Meeter, and R. P. L. S. Visser, "Medical Protection against Chemical Warfare Agents", SIPRI Books, Almqvist & Wiksell, Stockholm, 1976, Chapter 12.
(22) The possible involvement of syn/anti isomerism could be excluded, since the two isomers of 1 are eluted closely together from the high-performance LC column, in a position very different from that of the metabolite. **Note added in proof**: Our recent results indicate strongly that the metabolite is a conjugate of 1 and glucuronic acid.
(23) D. K. Meyers, *Biochim. Biophys. Acta*, **34**, 555 (1959).
(24) E. M. Cohen, P. J. Christen, and E. Mobach, *Proc. Kon. Ned. Akad. Wet., Ser. C.*, **74**, 113 (1971).
(25) E. Meeter, O. L. Wolthuis, and R. M. J. Van Benthem, *Bull. W.H.O.*, **44**, 251 (1971).
(26) J. H. Fleischer and L. W. Harris, *Biochem. Pharmacol.*, **14**, 641 (1965).
(27) E. J. Ariens, in "Drug Design", Vol. II, E. J. Ariens, Ed., Academic Press, New York, 1971, p 2.
(28) F. R. Sidell and W. A. Groff, *J. Pharm. Sci.*, **60**, 1224 (1971).
(29) L. Wolff, H. Kopitzsch, and A. Hall, *Justus Liebigs Ann. Chem.*, **333**, 6, (1904).
(30) R. G. Micetich, *Can. J. Chem.*, **48**, 2012 (1970).
(31) J. Goerdeler and H. Porrmann, *Chem. Ber.*, **95**, 627 (1962).
(32) L. P. A. De Jong, and B. I. Ceulen, *Biochem. Pharmacol.*, **27**, 857 (1978).
(33) P. J. Christen, Clinical Laboratory, Leeuwarden Hospitals, personal communication, 1974.
(34) H. R. Linder, H. P. Keller, and R. W. Frei, *J. Chromatogr. Sci.*, **14**, 234 (1976).
(35) H. P. Gelbke, T. H. Grell, and Gg. Schmidt, *Arch. Toxicol.*, **39**, 211 (1978).
(36) E. Meeter and O. L. Wolthuis, *Eur. J. Pharmacol.*, **4**, 18 (1968).