5-Methoxy-3-benzo[*b*]thiopheneacetic Acid (VIIb).—To 225 ml of 20% H_2SO_4 (prepared by adding 30 ml of concd acid to 267 ml of H_2O) was added 0.926 g (4.55 mmoles) of Vb. The mixture was refluxed with stirring for 48 hr after which time all of the nitrile had gone into solution. The solution was cooled and extracted with Et_2O (fraction A). The Et_2O was washed (10% $NaHCO_3$) and the aq phase carefully acidified (HCl) and extracted (Et_2O–fraction B). The Et_2O fractions were dried separately (Na_2SO_4) and evapd. No material was found in fraction A. Fraction B gave 0.735 g (73%) of off-white crystals. Sublimation afforded analytical material, mp 150–153°. *Anal.* ($C_{11}H_{10}O_3S$) C, H, O.

***N,N*-Dimethyl-5-methoxy-3-benzo[*b*]thienylacetamide (VIId).** To 40 ml of dry C_6H_6 containing 0.835 g (3.76 mmoles) of VIIb (not completely in solution) was added 2 g (15.7 mmoles) of oxalyl chloride and the greenish-yellow mixture was swirled and warmed to 55°. Within 60 min all material had gone into solution. The acid chloride remained as an oil after all volatile material had been removed under reduced pressure. Upon addition of 10 ml of C_6H_6 and then excess Me_2NH an exothermic reaction occurred. The C_6H_6 was then removed under reduced pressure and the residue dissolved in Et_2O, washed (H_2O), and dried ($MgSO_4$). Evaporation gave 0.89 g (95%) of amide which was purified *via* distillation at 140° (0.03 mm). The analytical material had mp 83–85°. *Anal.* ($C_{13}H_{15}NO_2S$) C, H, S.

***N,N*-Dimethyl-5-hydroxy-3-benzo[*b*]thienylacetamide (VIIc).** This material was made similarly to VIId. The analytical material (88%) was recrystd from C_6H_6, mp 163.5–165°. *Anal.* ($C_{12}H_{13}NO_2S$) C, H, S.

***N,N*-Diethyl-5-methoxy-3-benzo[*b*]thienylacetamide (VIIe).** This material was made similarly to VIId. The analytical material (81%) was distilled (130° 0.03 mm) to give a light yellow oil. *Anal.* ($C_{15}C_{19}NO_2S$)C, H, S.

***N*-Methyl-5-methoxy-3-benzo[*b*]thienylacetamide (VIIf).** This material was made similarly to VIId. The analytical material (94%) was sublimed (125°, 0.03 mm) to give white crystals, mp 137.5–139°; mol wt ($C_{13}H_{15}NO_2S$) calcd, 235.0667; found (mass spec), 235.0646.

5-Hydroxy-3-(β-*N,N*-dimethylaminoethyl)benzo[*b*]thiophene·HCl (VIIIa). To 0.79 g (3.35 mmoles) of VIIc in 175 ml of THF was added 0.54 g (14 mmoles) of LAH and the mixture was allowed to stir at 25° for 3.5 hr and then at reflux for 0.5 hr. Excess LAH was carefully destroyed (H_2O) and then 100 ml of dil acid was added to pH 5. THF was removed under reduced pressure and the solution was extracted (Et_2O) at pH 5, 8, 10, 12, and 14. The extracts were combined, dried ($MgSO_4$), and then saturated with HCl gas. Evaporation gave 0.73 g (85%) of a light yellow glass which was purified *via* distillation (185°, 0.01 mm). The material remained as a glass after purification. *Anal.* ($C_{12}H_{16}ClNOS$)C, H, Cl, S. A picrate was made by dissolving VIIIa in MeOH, adding a few drops of NH_3 and then boiling it with a saturated picric acid solution. Cooling afforded orange needles which were recrystd from MeOH, mp 199–200°. *Anal.* ($C_{18}H_{18}N_4O_8S$) C, H, N, S.

5-Methoxy-3-(β-*N,N*-dimethylaminoethyl)benzo[*b*]thiophene·HCl (VIIIb). To 1.54 g (6.2 mmoles) of VIId in 160 ml of dry Et_2O was added 4.5 g (12 *M*) of LAH and the mixture was allowed to reflux with stirring for 20 hr. Excess LAH was destroyed carefully (H_2O) and then 100 ml of 0.6 *N* NaOH was added. The whole mixture was filtered *via* suction and the trapped white salts were copiously washed with Et_2O. The NaOH solution was extracted (Et_2O) and all the extracts were then pooled, dried ($MgSO_4$), and satd with dry HCl. Initially the solution turned cloudy but as the HCl addition continued it became clear. Evaporation afforded 1.04 g (60%) of product. Sublimation (160°, 0.01 mm) gave 0.95 g of analytical material, mp 174–175°, white crystals. *Anal.* ($C_{13}H_{18}ClNOS$) C, H, S.

5-Methoxy-3-(β-*N,N*-diethylaminoethyl)benzo[*b*]thiophene·HCl (VIIIc). This material was made similarly to VIIIa except Et_2O was used as the solvent. Sublimation (160°, 0.01 mm) of the crude product gave 0.55 g (85%) of white crystals, mp 187–189°. *Anal.* ($C_{15}H_{22}ClNOS$) C, H, Cl, S.

Anti-Tremorine Test. The compounds were administered as suspensions in gum tragacanth at 100 mg/kg sc to groups of 5 male Evans albino mice. Thirty minutes later Tremorine tartrate (30 mg/kg) was given ip. After a further 20 min the mice were placed individually in the tremor recording chamber which is a circular "Perspex" box (11.25 cm diameter × 11.25 cm high), mounted on a thin strip of foam rubber on a metal stand. The box was divided diametrically so that the mice were restricted to one-half of the box. A Devices dynamometer type UFI was positioned underneath the edge of the box so that the vertical sensing lever was applied with a force of 30*g*. The dynamometer output was recorded *via* a DC2C preamplifier (range 2–5 mV) on a Devices recorder. Tremor was recorded for a period of 30 sec and anti-Tremorine activity expressed as a per cent of the tremor activity of a group of mice treated with Tremorine only.

Antinicotine Test. This test was also carried out in groups of 5 male Evans mice. The test compound was administered sc suspended in gum tragacanth, and the control groups received equiv vols of saline. Thirty minutes later the mice were given a further injection of 1 mg/kg of nicotine base iv (as a solution in saline of the bitartrate). The number of mice showing clear clonic convulsions within 1 min of injection were recorded.

Isothiazolecarboxaldoximes and Methylated Derivatives as Therapeutic Agents in Poisoning by Organophosphorus Compounds

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Received April 11, 1970

The reaction of Me tosylate with (*E*)-isothiazole-3-carboxaldoxime (**1a**, pK_a = 9.8) and with (*Z*)-isothiazole-5-carboxaldoxime (**1c**, pK_a = 8.6) led to the corresponding hydroxyiminomethyl-2-methylisothiazolium tosylates **2a** (pK_a = 7.6) and **2c** (pK_a = 2.6), respectively. Oxidation of 4-methylisothiazole with CrO_3 produced isothiazole-4-carboxylic acid, which was converted into isothiazole-4-carboxaldehyde by a Reissert reaction. The *E* and *Z* isomers of isothiazole-4-carboxaldoxime (**1b**, pK_a = 10.3 and 10.9, respectively) failed to give a 4-hydroxyiminomethyl-2-methylisothiazolium salt with a variety of methylating agents. In combination with atropine sulfate, the oximes (*Z*)-**1c** and **2a** are therapeutically active against poisoning with Sarin in mice, whereas **2a** is also active against Paraoxon. Both (*Z*)-**1c** and **2a** are less toxic than 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate (P_2S) in mice.

Oximes are potent reactivators of esteratic enzymes, inhibited by organophosphorus compounds.[1] Among these oximes, 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate (P_2S) and 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium) dibromide (TMB_4) were found to be particularly effective, both *in vitro* and *in vivo*.[1,2] The isosterism of the pyridine and isothiazole ring systems[3] suggested a study of the antidotal activity of hydroxyiminomethyl-2-methylisothiazolium salts. The preparation of these compounds from iso-

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(1) R. I. Ellin and J. H. Wills, *J. Pharm. Sci.*, **53**, 995 (1964).

(2) R. I. Ellin and J. H. Wills, *ibid.*, **53**, 1143 (1964).

(3) H. C. Longuet-Higgins, *Trans. Faraday Soc.*, **45**, 173 (1949).

thiazolecarboxaldehydes and preliminary data on their antidotal activity are reported.

Chemistry.—Isothiazole-3-carboxaldehyde was obtained from 3-methylisothiazole,[4] *via* the hydrolysis of 3-dibromomethylisothiazole.[5] Reaction of the aldehyde with NH_2OH in neutral aq solution gave isothiazole-3-carboxaldoxime(**1a**), configurationally pure according to its nmr spectrum. Since the original oxime was recovered in 80% yield from the alkaline hydrolysis of benzoylated **1a**, the *E* configuration[6] could be assigned[7] to **1a**. This assignment was confirmed by the presence of a sharp band at 3562 cm^{-1} ($CHCl_3$) in the ir spectrum of **1a**, indicating an OH group which is not associated with the neighboring ring N.[8] Attempts to isomerize (*E*)-**1a** under the influence of heat, uv radiation,[9] and concd aq HCl[10] failed. Methylation of (*E*)-**1a** proved to be difficult, probably due to the weakly basic character of the ring N (compare isothiazole,[11] pK_a −0.55). The least unsatisfactory result was obtained by treating (*E*)-**1a** with methyl tosylate,[12] which led to 3-hydroxyiminomethyl-2-methylisothiazolium tosylate(**2a**) in a 9.5% yield.

Oxidation of 4-methylisothiazole with CrO_3 gave isothiazole-4-carboxylic acid, which was converted into isothiazole-4-carboxaldehyde by a Reissert reaction.[4] Reaction of the aldehyde with NH_2OH in neutral aq solution led to a mixture of the geometric isomers of isothiazole-4-carboxaldoxime (**1b**). One of these isomers (mp 116°) was isolated from the mixture by dry column chromatography[13] on silica gel. The low-melting isomer (mp 82°) was obtained from reaction of the aldehyde with NH_2OH in strongly alkaline medium.[14] On comparing the nmr spectra of the two isomers (Table I), it was noted that the OH signals and those of the ring protons ortho to the hydroxyiminomethyl group were downfield for the high-melting isomer of **1b**, whereas the reverse effect was found for the CH of the hydroxyiminomethyl group. These chemical shift differences are characteristic for geometric isomerism in aromatic oximes[15] and allow the assignment of the *Z* configuration to the high-melting isomer of **1b**. Treatment of either (*E*)- or (*Z*)-**1b** with a variety of methylating agents gave unidentified decomposition products instead of the desired 4-hydroxyiminomethyl-2-methylisothiazolium salt. Attempts to prepare the latter compound *via* methylation of 4-(1,3-dioxolan-2-yl)isothiazole, followed by hydrolysis and reaction with NH_2OH,[16] were also unsuccessful.

Isothiazole-5-carboxaldehyde, prepared from isothiazol-5-yl Li and DMF,[17] afforded isothiazole-5-carboxaldoxime (**1c**) after treatment with NH_2OH in neutral aq solution. The nmr spectrum of **1c** revealed the presence of the *Z* and *E* isomers in a ratio of 9:1 (see Table I, compare **1b** for the assignment of configuration). Dry column chromatography on silica gel yielded the pure *Z* isomer of **1c**. Apparently, (*E*)-**1c** is rather unstable since the attempted isolation of this isomer resulted in an incomplete but rapid isomerisation. Reaction of (*Z*)-**1c** with methyl tosylate gave 5-hydroxyiminomethyl-2-methylisothiazolium tosylate (**2c**, 8.6% yield), characterized by its elemental analysis, uv, and nmr spectra.[18]

Enzymology.—The reactivating potency of the oximes listed in Table I was evaluated on bovine erythrocyte acetylcholinesterase (EC 3.1.1.7) inhibited with isopropyl methylphosphonofluoridate (Sarin). After removal of the excess of inhibitor, the reactivation of the enzyme by a 10^{-3} *M* solution of the oxime was followed for 20 hr at pH 7.5 (25°). Figure 1 shows that 3-hydroxyiminomethyl-2-methylisothiazolium tosylate (**2a**) restores the enzyme activity almost as fast and to the same

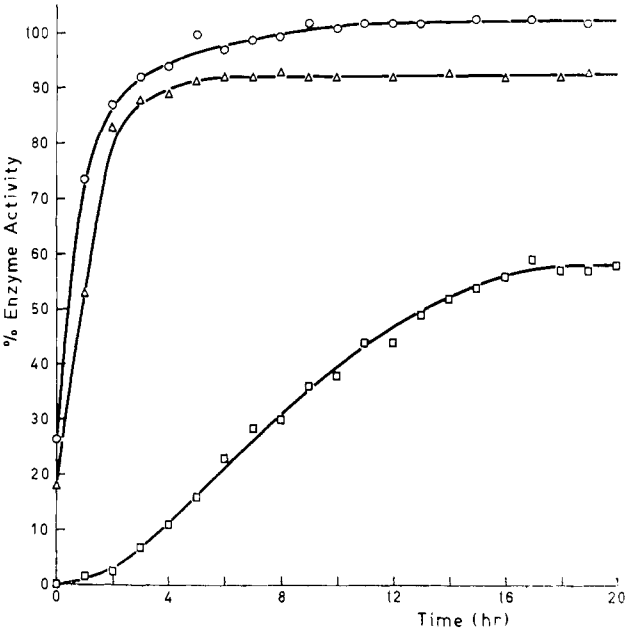

Figure 1.—Reactivation at pH 7.5 (25°) of AChE, inhibited with isopropyl methylphosphonofluoridate (Sarin). Reactivators (10^{-3} *M*): (○- - -) 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate (P_2S); (Δ- - -) 3-hydroxyiminomethyl-2-methylisothiazolium tosylate (**2a**); (□- - -) (*Z*)-isothiazole-5-carboxaldoxime (*Z*-**1c**).

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(6) The (*E*–*Z*) system of nomenclature for double-bond stereoisomers is used in this paper. See J. E. Blackwood, C. L. Gladys, K. L. Loening, A. E. Petrarca, and J. E. Rush, *J. Amer. Chem. Soc.*, **90**, 509 (1968).

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(11) Determined spectrophotometrically at 256 mμ.

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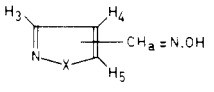
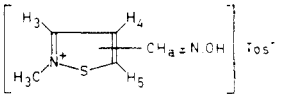
(18) To our knowledge, **2c** represents the most acidic oxime (pK_a 2.6, see Table I) which has been reported in the literature. The extremely large decrease in the pK_a of (*Z*)-**1c** on methylation (ΔpK_a 6.0, see Table I) will be the subject of a forthcoming paper, which will include molecular orbital calculations of the methylated isothiazole ring system. Methylation of the N or O of the hydroxyimino group of (*Z*)-**1c** with methyl tosylate can be excluded, since the condensation products of isothiazole-5-carboxaldehyde with *N*-methylhydroxylamine and with *O*-methylhydroxylamine have pK_a values 0.5 and −0.8, respectively. See also E. J. Poziomek, R. H. Poirier, R. D. Morin, and T. F. Page, *J. Org. Chem.*, **28**, 1411 (1963) and ref 16.

TABLE I

ISOTHIAZOLECARBOXALDOXIMES (**1**, X = S), METHYLATED DERIVATIVES (**2**), AND RELATED COMPOUNDS (**1**, X = O, NH)





Compd	X	Deriv	Configuration	Mp, °C	pK_a	Nmr absorption[a] (τ) H_3	H_4	H_5	H_a	OH	CH_3	Formula	Analyses[b]
1a	S	3-CH=NOH	E	76–77	9.8[c]		2.30[d]	0.92	1.69	−1.79		$C_4H_4N_2OS$	C, H, N, S
1b	S	4-CH=NOH	E	81–82	10.3	1.25		0.87	1.75	−1.25		$C_4H_4N_2OS$	C, H, N, S
1b	S	4-CH=NOH	Z	115–116	10.9	1.05		0.46	2.33	−1.81		$C_4H_4N_2OS$	C, H, N, S
1c	S	5-CH=NOH	Z	133–134	8.6	1.40[e]	2.29		1.79	−2.97		$C_4H_4N_2OS$	C, H, N, S
1c	S	5-CH=NOH[f]	E			1.40[g]	2.49		1.51	−1.88		$C_4H_4N_2OS$	
1d	O	5-CH=NOH[h]		121–123	9.3	1.31[h]	3.20[i]		1.67	−2.02		$C_4H_4N_2O_2$	C, H, N
1e	NH	3(5)-CH=NOH		107–109	10.5	2.29[j]	3.51		1.92			$C_4H_5N_3O$	C, H, N
2a		3-CH=NOH		191–192	7.6		2.20[k]	0.86	1.53		5.80	$C_{12}H_{14}N_2O_4S_2$	C, H, N, S
2c		5-CH=NOH		174–176	2.6	1.05[l]	2.25		1.12		5.92	$C_{12}H_{14}N_2O_4S_2$	C, H, N, S

[a] The nmr spectra were measured in DMSO-d_6 (Me_4Si). The investigations of Staab and Mannschreck [*Chem. Ber.*, **98**, 1111 (1965)] were used for interpretation of the nmr data. [b] All compounds analyzed correctly for the elements indicated within ±0.4% of the theoretical values. [c] The acid dissociation constants were determined potentiometrically in 0.1 *M* aqueous KCl, 25°. [d] Coupling constant $^3J_{4,5}$ = 4.8 cps, $^4J_{4,a}$ = 0.35 cps, $^5J_{5,a}$ = 0.75 cps. [e] Coupling constant $^3J_{3,4}$ = 1.8 cps. [f] Present as a minor amount (*ca.* 10%) in (*Z*)-**1c**, evidenced by the nmr absorptions at positions noted in this Table. [g] Prepared according to A. Ricca and G. Gandiano [*Atti Accad. Naz. Lincei Rend. Cl. Sci. Fis. Mat. Nat.*, **26**, 240 (1959)]. [h] Coupling constant $^3J_{3,4}$ = 2.4 cps, $^5J_{3,a}$ = 0.4 cps. [i] Coupling constant $^4J_{4,a}$ = 0.4 cps. [j] Coupling constant $J_{3(5),4}$ = 2.2 cps. The assignment of $H_{3(5)}$ is tentative, owing to the prototropy of **1e**. [k] Coupling constant $^3J_{4,5}$ = 6.0 cps. [l] Coupling constant $^3J_{3,4}$ = 3.0 cps.

extent as the isosteric 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate (P_2S). Isothiazole-5-carboxaldoxime ((*Z*)-**1c**) reactivates the enzyme slowly but to a significant extent (*ca.* 60%), whereas the remaining compounds from Table I give less than 15% reactivation in the course of 20 hr.

Since the decomposition of the inhibitor by oximes might contribute to their antidotal activity *in vivo*,[19,20] the rates of hydrolysis of Sarin (10^{-3} *M*) in the presence of a tenfold excess of the oximes (*Z*)-**1c**, **2a**, and P_2S were measured in 0.1 *M* aq KCl at 25°, pH 7.6. The observed rate constants (min^{-1}) were 0.37, 0.22, and 0.35 for the oximes (*Z*)-**1c**, **2a**, and P_2S, respectively. These results indicate that (*Z*)-**1c** and P_2S are equally effective for the breakdown of Sarin, whereas **2a** is slightly less active in this respect.

Pharmacology.—In view of the *in vitro* results, (*Z*)-**1c** and **2a** were selected for the investigation of their antidotal activity against poisoning with Sarin and Paraoxon. The data were compared with those for P_2S, which has been generally accepted as an antidote against poisoning with several organophosphorus compounds.[2] The acute ip toxicities of the oximes in mice were as follows (compound, LD_{50} read after 24 hr, 95% confidence limits): (*Z*)-**1c**, 295 (264–330) mg/kg; **2a**, 377 (369–384) mg/kg; P_2S, 227 (214–245) mg/kg. Hence, (*Z*)-**1c** and **2a** are less toxic than P_2S both on a weight and on a molar basis.

Table II shows that (*Z*)-**1c** gives a significant therapeutic effect against Sarin in mice, although less than P_2S, whereas (*Z*)-**1c** is not active against poisoning with Paraoxon. On the other hand, (*Z*)-**1c** is equally effective as P_2S against Sarin in rats: the same experiments as described in Table II for mice lead to LD_{50} (sc) values of Sarin in rats of 1.52 (1.40–1.66) mg/kg and 1.62 (1.45–1.81) mg/kg for the oximes (*Z*)-**1c** and P_2S, respectively, in combination with atropine sulfate.[21] Hence, the therapeutic activity of (*Z*)-**1c** is markedly dependent on the type of anticholinesterase used and on the animal species. Since limited quantities of the methylated oxime **2a** were available, this compound was only tested in mice. As shown in Table II, **2a** is not significantly less active than P_2S against Sarin, when reading the results after 24 hr. When comparing results after 48 hr, however, P_2S is found to be more active than **2a**. Finally, **2a** is equally active as P_2S against Paraoxon, this result being unchanged after 48 hr.

TABLE II

ANTIDOTAL ACTIVITY OF (*Z*)-ISOTHIAZOLE-5-CARBOXALDOXIME (*Z*-**1c**) AND OF 3-HYDROXYIMINOMETHYL-2-METHYLISOTHIAZOLIUM TOSYLATE (**2a**) AGAINST SARIN AND PARAOXON IN MICE

Treatment[a]	LD_{50}[b] (mg/kg) Sarin	Paraoxon
None	0.25 (0.24–0.26)	0.8 (0.7–0.9)
Atropine sulfate	0.30 (0.25–0.32)	3.5 (3.0–4.5)
(*Z*)-**1c** + atropine sulfate	0.45 (0.42–0.48)[c]	3.2 (2.8–3.6)
2a + atropine sulfate	0.56 (0.52–0.61)	9.7 (8.1–11.3)
	0.45 (0.35–0.50)[c]	
P_2S + atropine sulfate	0.64 (0.54–0.76)	9.8 (8.1–11.8)

[a] Atropine sulfate (37.5 mg/kg) and the oximes (*Z*-**1c**, 86.0 mg/kg; **2a**, 63.6 mg/kg; P_2S, 47.2 mg/kg) were administered ip, 1.5 min after intoxication. [b] Sarin and Paraoxon were administered subcutaneously in the neck. LD_{50} values were read after 24 hr, unless otherwise stated. Values in parentheses refer to 95% confidence limits. [c] After 48 hr.

Experimental Section

Melting points were determined on a Monoscop (Hans Bock, Frankfurt an der Main, West Germany) and are uncorrected. Nmr spectra were recorded on a Jeol C-60H spectrometer, using DMSO-d_6 as a solvent (Me_4Si). Ir spectra were obtained with a Grubb-Parsons GS 4 spectrometer. Uv spectra were recorded by means of a Zeiss RPQ 20A spectrometer in aqueous solution. Tlc was carried out on silica gel F254 (E. Merck) with C_6H_6–Me_2CO–EtOAc–concd NH_4OH (6:8:2:0.3, v/v);[22] the oximes

(19) W. D. Erdmann, *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol.* **263**, 61 (1969).

(20) J. H. Blanch, *J. Chem. Soc. B*, 1172 (1969).

(21) An LD_{50} (sc) value of 0.56 (0.39–0.91) mg/kg was found for Sarin, when the rats were only treated with atropine sulfate (37.5 mg/kg).

(22) B. Unterhalt, *Arch. Pharm.*, **300**, 38 (1967).

were visualized with uv light and by spraying with alcoholic $FeCl_3$ solution.[23] Dry column chromatography[13] was carried out on a nylon column filled with silica gel F254 (100 g). The positions of the products on the column were located with uv light (254 mμ). Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ± 0.4% of the theoretical values.

Isothiazole-3-carboxaldehyde.—The aldehyde was prepared from 3-methylisothiazole,[4] *via* the hydrolysis of 3-dibromomethylisothiazole:[5] bp 46–48° (0.7 mm) [lit.[5] bp 62–65° (9 mm)]. *Anal.* (C_4H_3NOS) C, H, N, S.

(*E*)-Isothiazole-3-carboxaldoxime (*E*-**1a**).—Isothiazole-3-carboxaldehyde (6.7 g, 59.2 mmoles) was added to a neutralized aq solution of $NH_2OH \cdot HCl$ (4.2 g, 60.4 mmoles). The mixture was homogenized with EtOH and heated for 30 min at 80°. After cooling to −60°, the product was filtered off and recrystallized from C_6H_6, which gave 6.7 g (88%) of pure (*E*)-**1a**: mp 76–77°; λ_{max} 269 mμ; 0.1 *N* NaOH, λ_{max} 330 mμ; $\nu_{OH}^{CHCl_3}$ 3562 cm^{-1}. Nmr spectroscopy and tlc showed that the product was isomerically pure.

(*E*)-*O*-Benzoylisothiazole-3-carboxaldoxime.—(*E*)-**1a** was benzoylated according to the method of Vermillion and Hauser,[10] mp 111–112°. *Anal.* ($C_{11}H_8N_2O_2S$) C, H, N, S. The benzoylated product (100 mg) was hydrolyzed in 2 *N* aq NaOH at room temperature in the course of 12 hr. After adjustment to pH 6.8, the reaction mixture was extracted with Et_2O. The combined extracts were evaporated after drying ($MgSO_4$). Recrystallization of the residue from C_6H_6 gave (*E*)-**1a** (44 mg, 80%), mp 73–74°. Nmr and ir spectroscopy showed that the oxime was identical with the product obtained from the reaction of isothiazole-3-carboxaldehyde with NH_2OH.

3-Hydroxyiminomethyl-2-methylisothiazolium Tosylate (**2a**).—A mixture of (*E*)-**1a** (215 mg, 1.68 mmoles) and methyl tosylate[12] (322 mg, 1.73 mmoles) was heated for 10 min at 115–120°. After rapid cooling to room temperature, the viscous reaction mixture was treated with Me_2CO (3 ml). The crude reaction product, crystallizing from the originally homogeneous solution after trituration, was filtered off and was further purified by recrystallization from MeOH–Me_2CO; yield 50.2 mg (9.5%) of **2a**: mp 191–192°; λ_{max} 290 mμ; 0.1 *N* NaOH, λ_{max} 330 mμ; purity 96.4% (potentiometric titration).

Isothiazole-4-carboxylic Acid.—An 80-g sample of 4-methylisothiazole[24] (0.81 mole) was added dropwise with stirring to concd H_2SO_4 (1.44 l.), maintaining the temperature below 30°. Powdered CrO_3 (208 g, 2.06 moles) was added portionwise to the stirred solution at 30° in the course of 6 hr, the stirring being continued for a further 12 hr. The reaction mixture was poured on to ice and extracted continuously (24 hr) with Et_2O. The extract was dried ($MgSO_4$) and evaporated to dryness under vacuum. The residue was recrystallized twice from H_2O, which gave the pure acid (57.9 g, 55.3 %); mp 162–163° (lit.[25] mp 162–164°). *Anal.* ($C_4H_3NO_2S$) C, H, N, S. The material was identical with that prepared by hydrolysis of 4-cyanoisothiazole.[25]

2-Cyano-1,2-dihydro-1-(isothiazole-4-carbonyl)quinoline.—Isothiazole-4-carbonyl chloride (25 g, 0.17 mole, obtained[25] from 23.1 g of isothiazole-4-carboxylic acid with $SOCl_2$) was added dropwise with vigorous stirring to a mixture of quinoline (22.0 g, 0.17 mole), KCN (19.2 g, 0.29 mole), CH_2Cl_2 (185 ml), and H_2O (90 ml) in the course of 3 hr. The mixture was additionally stirred at room temperature for 12 hr. After separation, the organic layer was washed successively with H_2O, 2 *N* aq HCl, H_2O, 2 *N* aq NaOH, and H_2O. Finally, the organic layer was dried ($MgSO_4$) and evaporated under vacuum, yielding the crude Reissert compound (24.0 g) as a brownish, semisolid substance. An analytical sample was obtained by recrystallization from cyclohexane–EtOH mp 124–126°. *Anal.* ($C_{14}H_9N_3OS$) C, H, N, S.

Isothiazole-4-carboxaldehyde.—A solution of the crude Reissert compound (23 g) in 9 *N* aq H_2SO_4 was steam distilled. After saturation with NaCl, the distillate (*ca.* 1.8 l) was extracted with Et_2O. The combined extracts were washed with aq Na_2CO_3, dried ($MgSO_4$), and evaporated under vacuum. Recrystallization of the residue from petroleum ether (bp 40–60°)–Et_2O (2:1, v/v) gave 4.7 g (24%, based on isothiazole-4-carbonyl chloride) of the pure aldehyde, mp 58–60° (lit.[26] mp 58°). *Anal.* (C_4H_3NOS) C, H, N, S.

4-(1,3-Dioxolan-2-yl)isothiazole.—The acetal was prepared from isothiazole-4-carboxaldehyde and ethylene glycol by the method of Bradsher and Parham,[27] yield 76%: bp 108–110° (2.5 mm); $n^{25}D$ 1.5387. *Anal.* ($C_6H_7NO_2S$) C, H, N, S.

(*Z*)-Isothiazole-4-carboxaldoxime (*Z*-**1b**).—Isothiazole-4-carboxaldehyde (2.0 g, 17.7 mmoles) was added to a neutralized aq solution of $NH_2OH.HCl$ (1.4 g, 20.1 mmoles). After heating for 30 min at 80°, the reaction mixture was concentrated and extracted with Et_2O. The combined extracts were dried ($MgSO_4$) and evaporated, which left 1.7 g (75.1%) of **1b**, melting range 50–90°. *Anal.* ($C_4H_4N_2OS$) C, H, N, S. The nmr spectrum of the product (see Table I) indicated a ratio of 3:2 for the *E* and *Z* isomers, respectively. The mixture (246 mg) was separated by means of dry column chromatography,[13] using C_6H_6–Et_2O (1:1, v/v) as a developing solvent. The *Z* isomer (lower R_f value) was cut out and extracted from the column with Et_2O. After evaporation of the solvent, the residue was recrystallized from CCl_4, giving (*Z*)-**1b** (72 mg), mp 115–116°. Both tlc and nmr showed the complete absence of the *E* isomer.

(*E*)-Isothiazole-4-carboxaldoxime (*E*-**1b**).—Isothiazole-4-carboxaldehyde (1.0 g, 8.8 mmoles) was added dropwise with stirring to a solution of $NH_2OH.HCl$ (0.75 g, 10.8 mmoles) in 5.5 *N* aq NaOH (4 ml) at −5 to −10° in the course of 30 min.[14] After additional stirring at −5° for 1 hr, the reaction mixture was neutralized with a saturated aq solution of NH_4Cl (6 ml) at −5°. The product was filtered off rapidly from the cold solution, dried under vacuum (P_2O_5) and purified by two recrystallizations from heptane–Et_2O (2:1, v/v), which gave 174 mg of (*E*)-**1b**, mp 81–82°. Both tlc and nmr showed the complete absence of the *Z* isomer.

Isothiazole-5-carboxaldehyde.—The aldehyde was prepared from isothiazole,[28] *via* the reaction of isothiazol-5-yl Li with DMF,[17] bp 28–29° (0.005 mm) [lit.[16] bp 95–98° (32 mm)]. *Anal.* (C_4H_3NOS) N, S.

(*Z*)-Isothiazole-5-carboxaldoxime (*Z*-**1c**).—Isothiazole-5-carboxaldehyde (1.0 g, 8.8 mmoles) was added to a neutralized aq solution of $NH_2OH.HCl$ (0.75 g, 10.8 mmoles). The mixture was homogenized with EtOH and heated for 30 min at 80°. After cooling to 0°, the product was filtered off and recrystallized from H_2O and C_6H_6, respectively, giving 1.0 g (88.6%) of **1c**, mp 125–128°. The nmr spectrum (see Table I) indicated the presence of (*Z*)-**1c** and (*E*)-**1c** in a ratio of 9:1. The isomeric mixture (500 mg) was separated by dry column chromatography[13] with C_6H_6–Me_2CO–EtOAc–concd NH_4OH (6:8:2:0.3, v/v) as a developing solvent. This procedure gave (*Z*)-**1c** (178 mg), mp 133–134°; λ_{max} 265 mμ; 0.1 *N* NaOH, λ_{max} 300 mμ. Both tlc and nmr showed the complete absence of (*E*)-**1c**.

5-Hydroxyiminomethyl-2-methylisothiazolium Tosylate (**2c**).—A solution of *Z*-isothiazole-5-carboxaldoxime (1.0 g, 8.8 mmoles) and methyl tosylate[12] (1.6 g, 8.8 mmoles) in C_6H_6 (5 ml) was heated for 1 hr at a bath temperature of 90°. The reaction product, which separated as a heavy oil, was dispersed in the solution with a stream of N_2 during the reaction. Next, the oil was separated from the reaction mixture and dissolved in Me_2CO (4 ml). Overnight, the product crystallized from the solution. A final purification was obtained by dissolving the product in a minimum of DMSO, followed by reprecipitation with Me_2CO, which gave **2c** (211 mg, 8.6%); mp 174–176°; 0.1 *N* H_2SO_4 λ_{max} 306 mμ; 0.1 *N* NaOH, λ_{max} 330 mμ; purity 97.5% (potentiometric titration).

Pyrazole-3(5)-carboxaldoxime (1e).—Pyrazole-3(5)-carboxaldehyde (1.9 g, 19.8 mmoles, prepared according to Bredereck, *et al.*[29]) was added to a neutralized solution of $NH_2OH \cdot HCl$ (1.5 g, 21.6 mmoles) in H_2O (80 ml). After heating for 30 min at 80°, the reaction mixture was concentrated under vacuum to 10 ml. Upon cooling, the oxime crystallized and was filtered off. The product was purified by recrystallization from MeOH–C_6H_6, which gave 350 mg of **1e**, mp 107–109°. Both nmr and tlc showed that the product was configurationally pure.

Kinetic Experiments. A. Reactivation of Inhibited Acetylcholinesterase (**AChE**).—Bovine erythrocyte acetylcholinesterase (EC 3.1.1.7, Winthrop Labor., Inc.) was completely phosphonyl-

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ated by incubation of a 25-ml solution, containing 1.5 mg of enzyme preparation/ml, with 25 ml of a 2×10^{-5} *M* solution of Sarin in a 6.6×10^{-3} *M* barbital-Na^+ buffer at 0° (pH 9.0) for 1 min. The excess of Sarin was removed by three extractions with 80-ml portions of ether (saturated with water). Reactivation was started by incubation of 40 ml of the remaining solution with 10 ml of a 5×10^{-3} *M* solution of the oxime in a 4×10^{-2} *M* phosphate buffer at 25° (pH 7.5). Enzyme activity was determined at hourly intervals, in 2-ml samples, by means of an automated pH-Stat procedure[30] using 3×10^{-3} *M* acetylcholine chloride as a substrate. Blanks for enzyme, enzyme with oxime, and inhibited enzyme were run simultaneously.

B. Hydrolysis of Sarin in the Presence of Oximes.—Into 5 ml of a 10^{-2} *M* solution of oxime in aq 0.1 *M* KCl, equilibrated at pH 7.6 and 25°, was introduced 5.5 μl of a solution of Sarin in *i*-PrOH. The final concentration of Sarin was approximately 10^{-3} *M*. The reaction rates were determined from the uptake of standard alkali by the reaction mixture, maintained at pH 7.6 by means of a Radiometer Autotitrator. A sufficient excess of oxime was used in order to provide first-order kinetics. Plots of log $(V_\infty - V_t)$ *vs.* time gave the pseudo-first-order rate constants. V_t and V_∞ are the amounts of alkali taken up at time t and after complete hydrolysis (after at least 8 half-lives), respectively. The rate of spontaneous hydrolysis of Sarin is negligible compared with the rate of reaction.

Pharmacological Procedures.—Female F_1 generation mice from a strain of our Laboratory, weighing 18–22 g, and female albino rats (Wistar), weighing 150–170 g, were used. Sarin and Paraoxon were administered subcutaneously in aq solution (mice, 10 ml/kg of body weight; rats, 2.5 ml/kg of body weight). Atropine sulfate was administered ip, 1.5 min after intoxication, in aq solution (mice, 10 ml/kg of body weight; rats, 1 ml/kg of body weight). The oximes were also administered ip, immediately after atropine sulfate. Due to the low solubility of (*Z*)-**1c** in H_2O, this oxime was administered in H_2O-DMSO [mice, 10 ml/kg, 6% (v/v) DMSO; rats, 2 ml/kg, 30% (v/v) DMSO]. The oxime **2a** was administered in aq solution to mice (10 ml/kg). See Table II (footnote *a*) for the administered doses of atropine sulfate, (*Z*)-**1c**, and **2a**. LD_{50} values were determined on 6 groups of 8 animals each and were calculated according to the method of Litchfield and Wilcoxon.[31]

Acknowledgments.—The authors are grateful to Professor E. M. Cohen and Miss E. Mobach, Medical-Biological Laboratory RVO-TNO, for the pharmacological data. We thank Mr. J. H. Keijer for the enzymological experiments. We are also indebted to Dr. F. Hübenett of Vickers-Zimmer A. G., Frankfurt an der Main, West Germany, for generous gifts of 4-methylisothiazole and to Dr. K. R. H. Wooldridge of May and Baker Ltd., Dagenham, Essex, England, for samples of 3-methylisothiazole.

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Perfluoroalkyl Carbonyl Compounds. 1. Perfluoroaldehyde and Perfluorocarboxylic Acid Derivatives

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Received January 16, 1970

As part of a search for biological activity in compounds containing strong electron-withdrawing groups directly attached to C=O, a series of derivatives of perfluoroalkyl aldehydes and perfluoroalkyl acids were subjected to biological evaluation. Interesting and diverse activities were found in derivatives of trifluoroacetaldehyde; in contrast, derivatives of longer chain fluorinated aldehydes and acids showed little activity. The active compounds which, it is suggested, may act by releasing CF_3CHO in the body of the test animal have effects on the endocrine glands and the CNS and reduce inflammation induced by carrageenin. Some of these show potent, but temporary, antifertility activity in male rats.

Carbonyl derivatives of 5-membered heterocyclic rings, substituted with strong electron-withdrawing groups, have been the subject of numerous publications.[1] Usually the electron-withdrawing entity has been NO_2, but compounds containing other electron-withdrawing substituents have also been studied. Recently Bambury, *et al.*,[2] have prepared a series of furfural derivatives containing the CF_3 group, and have found some anticoccidial activity in chickens.

As a result of our interest in this field, and consideration of unpublished work done in our own laboratories,[3] we came to the conclusion that the heterocyclic ring in such systems might not always be necessary, and that certain biological activity might be obtained in compounds where the electron-withdrawing group was directly attached to the carbonyl group.

The first series of such compounds investigated was the perfluoroalkyl aldehydes, such as trifluoroacetaldehyde. Most of the compounds described here are novel, and have been found to have unusual chemical and biological properties. However, we have included a few known compounds also, because these too have shown interesting biological activities which, to our knowledge, have not been described elsewhere.

The primary objective was to investigate the biological effects of the free CO compounds; however, the polar nature of these molecules (usually present as hydrates) could impair their absorption, and transport in the body to possible active sites. We felt that it was necessary to improve the biological transport of these compounds by masking the polarity of the CO group. Presumably this would increase fat solubility at the same time. It was also considered important that such derivatives should be of limited chemical stability so that they could be expected to revert to the free CO compounds under physiological conditions. These considerations led us to synthesize acetals and hemiacetals, Schiff bases, imidazolidines, thiazolidines, oxazolidines, and similar ring systems.

We were also interested in the biological effects of perfluoroalkyl carboxylic acids. The free acids, which

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