ated by incubation of a 25-ml solution, containing 1.5 mg of enzyme preparation/ml, with 25 ml of a 2×10^{-3} *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]

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Perfluoroalkyl Carbonyl Compounds. 1. Perfluoroaldehyde and Perfluorocarboxylic Acid Derivatives

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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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(1) For references see L. Doub, *Annu. Rep. Med. Chem.*, **1967**, 106 (1968).

(2) R. E. Bambury, H. K. Yakin and K. K. Wyckoff, *J. Heterocycl. Chem.*, **5**, 95 (1968).

(3) Mr. R. Sherlock of this Research Centre, is thanked for making available his unpublished results.

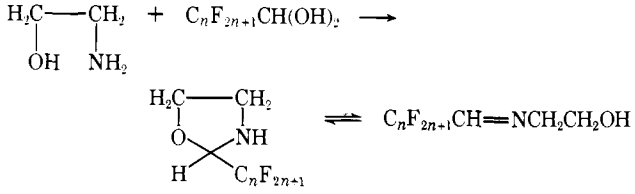
are too polar to be absorbed, were converted into trycyclic orthoesters[4] (**19–24**, Table I) which are nonpolar fat-soluble molecules, and should be readily absorbed following either oral or ip administration. We have found that these compounds are easily hydrolyzed, under mild conditions, to the free acids.

Chemistry.—Hemiacetals and acetals were prepared by standard methods.[5] Schiff bases were synthesized by the action of amines on the aldehyde hydrate in C_6H_6. These compounds were all hydrolyzed fairly readily to the aldehyde hydrate. The Schiff bases were the least stable, being completely hydrolyzed by H_2O at 20° in a few minutes. Efforts to isolate intermediate $C_nF_{2n+1}CH(OH)NHR$ by careful hydrolysis using **1** mole of H_2O in dry dioxane failed, in each case the only products recognized being the amine and the aldehyde hydrate.

The acetals and hemiacetals, while more stable than the Schiff bases, could still be hydrolyzed to the aldehyde hydrate, under relatively mild conditions. One compound, $C_2F_5CH(OEt)(OTs)$, was much more stable than the others.

The imidazolidines were prepared by treatment of the aldehyde hydrate with 1 mole of $H_2NCH_2CH_2NH_2$ in refluxing C_6H_6. They were susceptible to hydrolysis under mild conditions and decomposed when treated with HCl gas in dry Et_2O. Attempts to prepare derivatives of the NH of the imidazolidine ring failed, the ring fragmented and *N*-acyl derivatives of ethylenediamine were obtained.

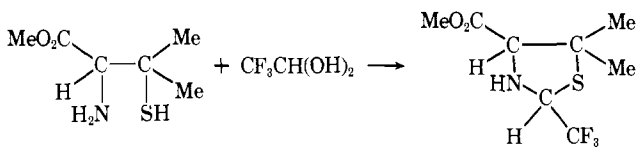
Oxazolidines were prepared by refluxing in C_6H_6 equimolar quantities of an α-amino alcohol and the aldehyde hydrate. The oxazolidines prepared were



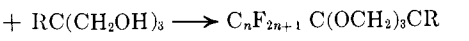
shown by nmr spectroscopy to be tautomeric mixtures of oxazolidine and Schiff Base.[6] The proportion of isomers present at equilibrium in each compound is detailed in Table I.

The oxazolidines had chemical properties similar to the imidazolidines, were easily hydrolyzed, did not give stable HCl salts, and decomposed on attempted alkylation or acylation.

One example of a thiazolidine was prepared by treating penicillamine methyl ester with trifluoroacetaldehyde hydrate (**18**, Table I).



Trycyclic ortho esters were formed when the perfluorocarboxylic acid was refluxed with triols in C_6H_6 or PhMe.[4]



The ortho esters are volatile compounds, insoluble in H_2O, but soluble in organic solvents. They were rapidly hydrolyzed to the acid in a few minutes by treatment with H_2O–Et_2O. Slow hydrolysis also took place in moist air.

Biological Activity.—The observed biological activities of novel compounds are described in Table I, and the known compounds in Table II.[7-9]

Effects on testes and thymus weight were noted in some of the compounds, and it is clear that the most active ones (**7**, **10**, **12**, and **16**) contain the CF_3 group, and can release CF_3CHO. Compounds containing C_2F_5 and C_3F_7 groups which can release the higher perfluoroaldehydes were much less active (**8**, **9**, **15**). To test this hypothesis $CF_3CH(OH)_2$ and some of its hemiacetals were examined and these compounds (**25**, **26**, **27**) were found to have a marked effect. Longer chain perfluoroaldehydes and their acetals (**29**, **30**, **31**) were inactive. Compounds **26** and **27** were found to be male antifertility agents. Male rats dosed with these compounds at daily 50 and 25 mg/kg for 4 weeks became infertile, but 2–3 months after the dosage was discontinued, fertility was regained. Histological examination showed total suppression of spermatogenesis but there was no evidence of damage to testicular tissues.

Most of the compounds exerted depressant effects on the CNS. Some compounds also showed activity on the carrageenin-induced edema test; a representative member of the series, **13**, when submitted to the adjuvant-induced arthritis test, produced no useful effect. The derivatives of perfluoroalkyl acids were mostly inactive apart from some response on the carrageenin screen.

All the compounds described in this paper were tested *in vitro* using standard screening methods, against a range of fungi and typical Gram-positive and Gram-negative bacteria. They were also tested for activity against experimental infections of schistosomiasis, amoebiasis, trypanosomiasis, and trichomoniasis in laboratory animals. No activity was shown against any of these organisms *in vitro* or *in vivo*.

Experimental Section

Microanalytical figures for novel compounds given in Table I agree with the theoretical values to within ±0.4%. The structures of all compounds in Table I were confirmed by ir and nmr spectroscopy. Known compounds mentioned in Table II were prepared by established methods. Melting points are uncorrected and were measured on a Kofler microscope hot stage.

Some representative preparations are detailed.

Method A. 2,2,3,3,3-Pentafluoro-1-ethoxy-1-tosyloxypropane (1).—Pentafluoropropionaldehyde hemiacetal (3.9 g, 0.02 mole) was dissolved in dioxane (20 ml). *p*-TosCl (4.0 g, 0.022 mole) and Et_3N (2.2 g, 0.022 mole) were dissolved in another portion of dioxane (20 ml), the two solutions were mixed and left for 18 hr at 25°. $Et_3N \cdot HCl$ was removed by filtration, and the filtrate was concd to give an oil which was distd to give a clear oil (2.0 g, 29%), bp 104–106° (2 mm).

Method B. 2,2,2-Trifluoroethylidonecyclohexylamine (2).—Trifluoroacetaldehyde monohydrate (5.8 g, 0.05 mole) and cyclohexylamine (5.5 g, 0.055 mole) were heated together in C_6H_6

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Table I
Novel Compounds

No.	Structure	% ring form	Method	Yield, %	Bp (mm) or mp, °C	Formula	Analyses	LD50[a] Oral	LD50[a] Ip	CNS[b] activity	Antiinflammatory[c]	Endocrine effects[d] T	Th	C	B wt	L
1	$C_2F_5CH(OEt)$ (*O*-Tosyl)		A	29	104–106 (2)	$C_{12}H_{13}F_5O_4S$	C, H, F		580		0	...	...	...	...	...
2	$CF_3CH{=}NC_6H_{11}$		B	56	144–146 (760)	$C_8H_{12}F_3N$			490	Depression	0	...	...	...	...	...
3	$C_2F_5CH{=}NC_6H_{11}$		B	49	148–150 (760)	$C_9H_{12}F_5N$			>1000	Depression	9	...	...	...	...	...
4	$C_2F_5CH{=}NC(CH_3)_3$		B	32	90–91 (760)	$C_7H_{10}F_5N$			>1000	Depression	9	...	...	...	...	...
5	$CF_3CH{=}N$—Ad Ad = 1-adamantyl		B	34	50	$C_{12}H_{16}F_3N$	C, H, F, N		>1000	Depression	65	...	...	...	...	...
6	$C_2F_5CH{=}N$—Ad		B	82	111 (18)	$C_{13}H_{16}F_5N$			>1000	Excitation—low doses; depression high doses	0	...	...	...	...	...
7	$HNCH_2CH_2NHCH{-}R$; R = $CF_3 \cdot 0.5H_2O$ ⟶		C	79	72–75	$C_4H_7F_3N_2 \cdot 0.5H_2O$	C, H, F, N	600	350	Depression	32	56	73	...	94	...
8	R = C_2F_5		C	76	85	$C_5H_7F_5N_2$	C, H, F, N	800	700	Depression	14	...	...	77	85	136
9	R = C_3F_7 ⟶		C	62	68	$C_6H_7F_7N_2$	C, H, F, N	600	200	Depression	19	...	...	...	85	...
10	$HNCH_2CH_2OCH{-}R$ ⇌															
	$RCH{=}NCH_2CH_2OH$, R = CF_3	100	D	49	138–140 (760)	$C_4H_6F_3NO$	C, H, F, N	600	700	Depression	28	65	83	82	...	110
11	R = CF_3,4-Et	90	D	63	148–152 (760)	$C_6H_{10}F_3NO$	C, H, F, N	>1600	1000	Depression	6	...	...	...	...	...
12	R = CF_3,5-Me	83	D	52	132–135 (760)	$C_5H_8F_3NO$	C, H, N	>1600	1000	Depression	0	65	77	79	...	...
13	R = CF_3,4,4-Me_2	76	D	45	134–138 (760)	$C_6H_{10}F_3NO$	C, H, N	1000	1000	Depression	49	...	...	...	...	...
14	$HNCH_2CH_2OCHR$, R = CF_3; 4-methyl-5-phenyl	60	D	61	102–106 (4)	$C_{11}H_{12}F_3NO$	C, H, N	800	600	Convulsions	0	x	x	x	x	x
15	R = C_2F_5	99	D	47	132–134 (760)	$C_5H_6F_5NO$	C, H, N	>1600	1000	Depression	18	...	...	75	...	130
16	$HN(CH_2)_3OCHCF_3$ ⇌ $CF_3CH{=}N(CH_2)_3OH$	92	D	56	130–135 (760)	$C_5H_8F_3NO$	C, H, N	600	400	Depression	22	59	68	77	...	...
17	$HN(CH_2)_5OCHCF_3$ ⇌ $CF_3CH{=}N(CH_2)_5OH$	85	D	52	100–110 (4)	$C_7H_{12}F_3NO$	C, H, N	>1600	>800	Depression	20	...	...	...	...	...
18	$(CH_3)_2CSCH(CF_3)NHCHCOOH$		E	44	68–72 (30)	$C_5H_{12}F_3NO_2S$	C, H, N	>1600	>800	Depression	0	x	x	x	x	x
	R R_1															
19	$RC(CH_2O)_3CR_1$		F	71	84	$C_8H_{11}F_3O_3$	C, H, F	600	750	Depression	37	...	...	...	...	...
	C_2H_5 CF_3															
20	C_3H_7 CF_3		F	43	125	$C_9H_{13}F_3O_3$	C, H	800	600	Depression	23	...	...	...	...	...
21	CH_3 C_2F_5		F	49	136	$C_8H_9F_5O_3$	C, H	>1600	>800		0	...	...	...	...	...
22	C_2H_5 C_2F_5		F	59	121	$C_9H_{11}F_5O_3$	C, H	>1600	>800		23	...	...	...	...	...
23	CH_3 C_3F_7		F	39	93	$C_9H_9F_7O_3$	C, H	>1600	>800		0	...	...	...	...	...
24	C_2H_5 C_3F_7		F	55	92	$C_{10}H_{11}F_7O_3$	C, H	>1600	>800		12	...	...	...	...	...

[a] The LD_{50} was determined in mice. [b] CNS effects were observed in normal mice. [c] Figures refer to the per cent reduction in edema, induced by carrageenin, relative to controls.[9] [d] Endocrine tests were performed on rats given 12 daily doses of 50 mg/kg subcut: T = testes; Th = thymus; C = cholesterol levels; B wt = body weight; L = liver; x = results not available; ... = not significantly different from controls. Figures refer to per cent weight relative to controls.

TABLE II
KNOWN COMPOUNDS

No.	Structure	Formula	LD50[a] Po	LD50[a] Ip	CNS[b] activity	Anti-inflammatory[c] activity	Endocrine effects[d] T	Th	C	B wt	L
25	$CF_3CH(OH)_2$	$C_2H_3F_3O_2$[7]	600	600	Depression	x	56	60	...	84	...
26	$CF_3CH(OH)(OCH_3)$	$C_3H_5F_3O_2$[5]	750	550	Depression	x	46	54	...	...	...
27	$CF_3CH(OH)(OC_2H_5)$	$C_4H_7F_3O_2$[5]	600	600	Depression	73	45	...	77	...	...
28	$CF_3CH(OC_2H_5)_2$	$C_6H_{11}F_3O_2$[5]	>1000	600	Depression	x	...	...	...	...	...
29	$C_2F_5CH(OH)_2$	$C_3H_3F_5O_2$[7]	600	600	Depression	x	...	...	...	...	...
30	$C_2F_5CH(OH)(OC_2H_5)$	$C_5H_7F_5O_2$[5]	1200	800	Depression	x	...	...	...	...	...
31	$CF_3(CF_2)_6CH(OH)_2$	$C_8H_3F_{15}O_2$[7]	>1000	>800	Depression	x	...	...	...	...	...
32	$CF_3CH(OCOCH_3)_2$	$C_6H_7F_3O_4$[8]	>1000	>1000		x	...	...	...	...	...

[a] The LD_{50} was determined in mice. [b] CNS effects were observed in normal mice. [c] Figures refer to the per cent reduction in edema, induced by carrageenin, relative to controls.[9] [d] Endocrine tests were performed on rats given 12 daily doses of 50 mg/kg subcut: T = testes; Th = thymus; C = cholesterol levels; B wt = body weight; L = liver; x = results not available; ... = not significantly different from controls. Figures refer to per cent wt relative to controls.

(50 ml). H_2O (1.8 g, 0.1 mole) was collected in a Dean–Stark trap in 2 hr. Excess C_6H_6 was removed by evapn and the residual oil was distd to give a colorless oil (5.0 g, 56%), bp 144–146°.

Method C. 2-Trifluoromethylimidazolidine (7).—Ethylenediamine (6.0 g, 0.1 mole) and trifluoroacetaldehyde hydrate (12 g, 0.104 mole) were refluxed in C_6H_6 (100 ml) for 2 hr. H_2O (2.5 ml) was collected in a Dean–Stark trap during this time. Solvent was removed by evapn under reduced pressure leaving an oil, which was dissolved in $CHCl_3$ and chromatographed on neutral Al_2O_3. Concn of the eluted material gave **7** as a white crystalline solid (11.8 g, 79%), mp 72–75°. Elemental analyses corresponded to a hemihydrate $C_4H_7F_3N_2 \cdot 0.5H_2O$, and this structure was supported by nmr and mass spectral measurements.

Method D. 2-Trifluoromethyl-4-ethyloxazolidine (11).—Trifluoroacetaldehyde hydrate (13.0 g, 0.11 mole) and 2-amino-1-butanol (8.9 g, 0.1 mole) in C_6H_6 (100 ml) were heated under reflux for 1.5 hr. A Dean–Stark trap was connected and the reaction was allowed to proceed until H_2O no longer collected in the trap. H_2O (3.6 g, 0.2 mole) was collected in 24 hr. C_6H_6 was removed by evapn under reduced pressure and the oily residue dissolved in $CHCl_3$ was purified by passage through a short neutral Al_2O_3 column. The purified solution was concd to give an oil which was distd to give a colorless oil (10.65 g, 63%), bp 148–153°.

Method E. Methyl 2-Trifluoromethyl-4,4-dimethylthiazolidine-5-carboxylate (18).—Trifluoroacetaldehyde hydrate (12 g, 0.104 mole) and penicillamine Me ester (16.3 g, 0.1 mole) in C_6H_6 (150 ml) were refluxed for 18 hr under a Dean–Stark trap. Solvent was removed by evapn to leave an oil which was distd to give a colorless oil (10.6 g, 44%), bp 68–72° (30 mm).

Method F. 1-Trifluoromethyl-4-ethyl-2,6,7-trioxabicyclo[2.2.2]octane (19).—Trifluoroacetic acid (12 g, 0.105 mole) and 2-ethyl-2-hydroxymethylpropane-1,3-diol (13.4 g, 0.1 mole) in *p*-xylene (100 ml) were refluxed together under a Dean–Stark trap for 6 hr. Xylene was removed by evaporation under reduced pressure and the residue in $CHCl_3$ was purified by passage through neutral Al_2O_3. The $CHCl_3$ soln was then concd to give a solid which was recrystd from petr ether (40–60°). Compd **19** was obtained as a white crystalline solid, mp 84° (15.0 g, 71%).

Acknowledgment.—We wish to express our appreciation to members of our Spectroscopy Laboratory for the determination of physicochemical properties and to many members of our Biology Department for carrying out the biological tests reported in this paper.

Perfluoroalkyl Carbonyl Compounds. 2. Derivatives of Hexafluoroacetone

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Seventeeen derivatives of hexafluoroacetone (HFA), 9 of them novel compounds, have been synthesized and subjected to chemical and biological study. Some interesting chemical properties of these compounds are described. Significant activity against the parasite *Trypanosome rhodesiense* was found for HFA and some of its simple derivatives. It is suggested that HFA is the active agent, and that its derivatives owe their activity to the release of HFA *in situ*. Other biological activities were also observed.

Following our work on the perfluoroalkyl aldehydes[1] we now present a study of hexafluoroacetone. The objective was the examination of the biological properties of masked hexafluoroacetone (HFA), and as a consequence some simple derivatives of the ketone, with limited stability have been synthesised. The arguments presented in part 1[1] for this approach are also relevant to the present study.

The systems chosen for study were (a) $X(CH_2)_nYC(CF_3)_2$ where X or Y = O, S, NH and (b) $(CF_4)_2C(OH)R$ where R = N< or CH<

Chemistry.—The imidazolidines **1** and **2** (Table I) were prepared by the reaction of the diamine with hexafluoroacetone or with hexafluoroacetone sesquihydrate (HFAS). They were isolated as crystalline solids with 1 mole of H_2O firmly bound. This could not be removed by drying or by sublimation *in vacuo*. An nmr study of **1** showed that an equilibrium between the

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