C=C); 1475, 1430, 1420, 1365, 835, 810, 640, 600 (unassigned); τ in ppm: 7.43 (SCH_3), 2.94 d and 1.8 d (H_5 and H_6), 1.7 (H_2).

Anal. Calcd for $C_7H_7N_3S \cdot {}^7/_8H_2O$: C, 46.48; H, 4.88; N, 23.23. Found: C, 46.43; H, 4.76; N, 23.26.

3-β-D-Ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XV).[5]—A solution of 7-chloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XII, 70 mg, 0.25 mmoles) in ethanol–water (5:10 ml) was hydrogenated at atmospheric pressure in the presence of MgO (9.8 mg, 0.2 mmole) and 5% Pd-C catalyst (10 mg). After the theoretical amount of hydrogen had been consumed, the catalyst and MgO were removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in water (1 ml) and filtered through dry Celite and the filtrate was refrigerated overnight. The crystals that formed were collected by filtration, washed, and dried *in vacuo;* yield 28 mg (45%), mp 226° (lit.[5] 220–222°), $[\alpha]^{23}D$ −78.0 ± 0.5° (0.99 g/100 ml of methanol). Thin layer chromatography using chloroform–methanol (9:1) as the eluent showed one spot which gave a positive Schiff-metaperiodate test: λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—236 (5.0), 274 (9.9), 281 (8.7); pH 7—244 (5.1), 277 (sh), 281 (8.5), 287 (6.6); pH 13—246 (4.8), 277 (sh), 281 (8.2), 286 (sh); [lit.[5] λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 0.5—236 (5.6), 275 (10.1), 281 (8.6); pH 5.66—243 (4.9), 281 (8.5), 287 (6.6)]; $\bar{\nu}_{max}$ in cm^{-1}: 3340, 3240, 3125, 2980, 2940, 2920, 2860 (OH, CH); 1595, 1580 (C=C, C=N); 1130, 1115, 1105, 1075, 1050 (CO-).

Acknowledgment.—The authors are indebted to Mr. Jerry Frye for technical assistance, to Dr. W. J. Barrett and members of the Analytical Section of this Institute who performed the spectral and analytical determinations, and to Miss E. A. Dulmadge for the cytotoxicity data reported herein.

Potential Carcinolytic Agents.[1] III. Fluoronitrogen Mustard Analogs of Cyclophosphamide[2]

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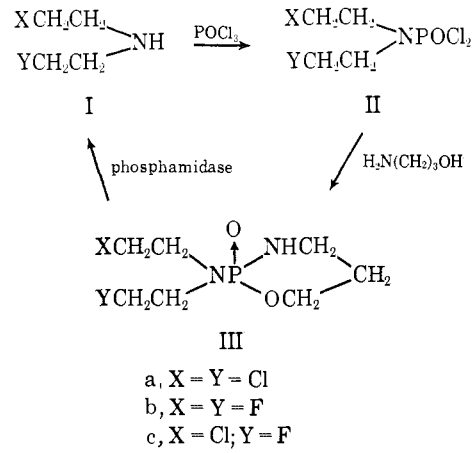
Received November 13, 1965

Two fluoro analogs of cyclophosphamide, 2-[bis(2-fluoroethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide and 2-[(2-chloro-2′-fluorodiethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide, were synthesized. These compounds and their precursors displayed little, if any, antitumor activity against rodent tumors.

Cyclophosphamide (IIIa) is one of the most effective biological alkylating agents for treating certain experimental rodent malignancies.[3] However, rather disappointing results were reported in treating neoplastic diseases in man with this agent.[4] Many structural modifications[5] of this compound have not produced any superior antitumor agents against animal tumors.

Recently a number of Russian investigators[6] have reported that some fluoroanalogs of nitrogen mustard derivatives exhibit antitumor properties (*e.g.*, 5-(2-chloro-2′-fluorodiethylamino)-6-methyluracil, see also ref 7). Also, Pettit and Smith[8] found that bis(2-fluoroethyl)amine hydrobromide inhibited the growth of Walker 256 carcinosarcoma (40% by 0.6 mg/kg, but the therapeutic index was less than 1). Our investigation on the mechanism of action of fluoroethylamines demonstrated that these compounds act, *in vitro*, like the corresponding chloroethylamines through the formation of an aziridinium intermediate but at a slower rate.[9] It was therefore of interest to examine if the oxazaphosphorinane moiety of cyclophosphamide (IIIa) could act as a carrier of the cytotoxic fluoro nor-nitrogen mustards Ib and c in transporting IIIb, IIIc, or an activated product derived from them[10] to the tumor site. Hydrolysis, catalyzed by phosphamidase, could then produce the active biological alkylating agents Ib and c.



a, X = Y = Cl
b, X = Y = F
c, X = Cl; Y = F

Straight-chain ω-fluoroalkylamines with an even number of carbon atoms are very toxic because they are oxidized by monoamine oxidases to precursors of

(1) Previous paper: Z. B. Papanastassiou and R. J. Bruni, *J. Org. Chem.*, **29**, 2870 (1964). This work was sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-4360.

(2) Cyclophosphamide: 2-[bis(2-chloroethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide.

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fluoroacetic acid.[11] Since most tumors are unable to utilize fluoroacetate in a "lethal" synthesis of fluorocitrate as the liver does,[12] any antitumor effect of IIIb and c could have been attributed to their latent biological alkylating action. On the other hand, high toxicity to the host and absence of any antitumor action could support the hypothesis that splitting of the mustard moiety occurs in the liver as the studies of Foley and co-workers[13] have indicated for cyclophosphamide IIIa (see also ref 14). The antitumor activity of the compounds is summarized in Table I.

TABLE I[a]
BIOLOGICAL ACTIVITY OF FLUORO MUSTARDS

Compd	KB[b]	L1210[c]	Ca755,[d] %	LD_{50}, mg/kg[e]	DA,[f] %	LD_{50}, mg/kg[e]	DX[g]	WA-256[h]
Ib·HBr	NA	NA	50[i]	2	NA		NA	
Ic·HCl	28	NA	70[j]	3	150[k]		NA	
IIb	NA	NA	50[l]	4	NA		NA	
IIc	16	NA	NA	10	NA		NA	
IIIb	NA	NA	50[m]	30	NA		NA	NA
IIIc	31	NA	NA		140[n]	30		

[a] The compounds were evaluated for the Cancer Chemotherapy National Service Center and complete data will be published in a future Cancer Chemotherapy Screening Data Supplement to *Cancer Research* (for a description of the antitumor assays used, see ref 3). We wish to thank Dr. Philip S. Thayer and Mr. I. Wodinsky, Arthur D. Little, Inc., for screening the compounds, and Dr. George R. Pettit for permitting us to quote the screening results of Ib (see also ref 8). NA means no activity. [b] ID_{50}, μg/ml. [c] Lymphoid leukemia L1210. [d] Adenocarcinoma 755 tumor weight (treated/control × 100); BDF1 mice given eleven daily intraperitoneal injections starting 1 day after tumor implantation; the animals were sacrificed on the twelfth day. [e] LD_{50} values are approximate doses in tumor-bearing animals. [f] Dunning leukemia (ascites) survival time (treated/control × 100); Fisher rats were given five daily intraperitoneal injections starting 1 day after tumor implantation. [g] Dunning leukemia (Cytoxan resistant). [h] Walker 256 (intramuscular). [i] At 0.6 mg/kg. [j] Over a range of 1–8 mg/kg. [k] Over a range of 0.25–2 mg/kg. [l] Over a range from 0.16–2.5 mg/kg. [m] Over a range of 0.94–15 mg/kg. [n] At 12.5 mg/kg.

It is interesting to note that all fluorochloro mustards (Ic, IIc, and IIIc) are cytotoxic in the KB cell culture [cyclophosphamide (IIIa) is inactive in this test system] and two of them exhibit some weak activity against Dunning leukemia, a tumor very sensitive to IIIa. The difluoro mustards (Ib, IIb, and IIIb) show some weak but not reproducible activity in Ca755 and no activity in the KB cell culture. Surprisingly, the one-armed fluoro mustard ($FCH_2CH_2NH_2 \cdot HCl$) is very active with an ID_{50} of 4 μg/ml in the KB cell culture system;[15] since aziridine displays a similar activity and methyl fluoroacetate or potassium fluoride are inactive in this assay system,[15] a possible explanation for the activity of 2-fluoroethylamine is its conversion to aziridine. On the other hand, our nmr studies[9] indicate that only the expected twofold statistical difference exists in the rate of cyclization of 2-fluoroethylamine and bis(2-fluoroethyl)-amine to the corresponding aziridinium salts.

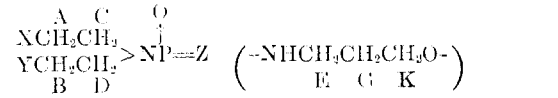
From these results it appears that, *in vivo*, IIIb and c do not act as analogs of cyclophosphamide, being more toxic and much less active as antitumor agents.

Compounds IIIb and c were synthesized by known methods.[3] They were oils which did not form crystalline hydrates as cyclophosphamide does. The synthesis of the starting materials Ib and c was described in our preceding publication.[1]

The precursors IIb and c were prepared in low yields by heating the amines Ib and c with phosphorus oxychloride. However, during the reaction redistribution of the halogens occurred (as determined by nmr spectroscopy) and the desired isomers could be separated by fractionation through a Podbielniak Whirling Heli-band distillation apparatus of *ca.* 50 theoretical plates. The redistribution of the halogens could be avoided by running the reaction of Ib with $POCl_3$ at low temperature in the presence of triethylamine.

The nmr spectra have been quite useful in both qualitative identification and quantitative estimation of I–III, and especially for the fluorine-containing compounds in which the characteristic splitting and downfield shift due to fluorine substitution affords a simple indirect means of fluorine analysis which is otherwise difficult to obtain. However, the data presented in Table II indicate the degree of complexity in the spectra

TABLE II
NMR SPECTRA[a] OF β-HALOETHYLPHOSPHAMIDATES



IIa, X = Y = Cl; Z = Cl_2
b, X = Y = F; Z = Cl_2
c, X = F; Y = Cl; Z = Cl_2
IIIa, X = Y = Cl; Z = $-NH(CH_2)_3O-$
b, X = Y = F; Z = $-NH(CH_2)_3O-$
c, X = F; Y = Cl; Z = $-NH(CH_2)_3O-$

Proton	IIa	IIb[b]	IIc	IIIa	IIIb[c]	IIIc
δA	3.7 (m)	4.60 (dt)	4.57 (dt)	3.5 (m)	4.53 (dt)	3.5 (m)
δB						
δC		3.63 (2dt)	3.70 (m)		3.42 (2dt)	
δD						
δE					3.0–3.9 (m)	
δG				1.85 (t)	1.82 (q)	1.85 (q)
δK				4.43 (t)	4.53 (2dt)	4.43 (t)

[a] t = triplet, dt = pair of triplets, 2dt = two pair of triplets, q = quintet, m = center of multiplet; in parts per million from TMS in $CDCl_3$. [b] J_{FA} = 47 cps, J_{FC} = 25 cps, J_{PC} = 16 cps, J_{AC} = 5 cps. [c] J_{FA} = 47 cps, J_{FG} = 25 cps, J_{PC} = 12 cps, J_{PE} = 15 cps, J_{AC} = 5 cps, J_{EG} = 5 cps, J_{GK} = 5 cps.

of these compounds due to both ^{19}F–H and ^{31}P–H spin–spin splitting, as well as the H–H splitting. The absorption of the haloethyl group in compounds II and III is quite similar to that reported[9] for I. Inconsistencies, perhaps due to diamagnetic shielding effects, in the difference of δ values for the A and B protons in the haloethylamine, its hydrochloride salt, and the phosphoramidates II and III make it difficult to assess the inductive effect of the dichlorophosphoryl and oxazaphosphorinane groups.

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Experimental Section[16]

N,N-Bis(2-fluoroethyl)phosphoramidic Dichloride (IIb). A.—A suspension (10 g, 0.069 mole) of Ib·HCl[1] in 49 ml of redistilled $POCl_3$ was stirred and heated in an oil bath at reflux temperature for 40 hr. Excess $POCl_3$ was removed in a rotary evaporator and the product was distilled under vacuum through a 10-cm Vigreux column to give, after a forerun, two fractions: 5.2 g, bp 100–110° (1.5 mm), $n^{25}D$ 1.4535; and 8.2 g, bp 110–120° (1.5 mm), $n^{25}D$ 1.4670.

The same experiment was repeated but heating of the mixture was stopped after 18 hr. The product (15 g) was distilled at 110–125° (1.5 mm), giving two fractions (of about equal weight), $n^{25}D$ 1.4590 and 1.4696. The products obtained from the two runs were combined and redistilled in a Podbielniak Series 3400, Mini-cal Whirling Heli-band distillation apparatus at *ca.* 1-mm pressure and a reflux ratio of 50:1. The following fractions were obtained: (a) bp 73–86° (4 g), $n^{25}D$ 1.4225; (b) bp 86–89° (8 g), $n^{25}D$ 1.4545; (c) bp 89–92° (4 g), $n^{25}D$ 1.4580; (d) bp 92–96° (5 g), $n^{25}D$ 1.4595; (e) bp 96–98° (5 g), $n^{25}D$ 1.4845. Assay by nmr spectroscopy indicted that fractions c and d were mostly the desired compound IIb: ν_{max}^{NaCl} 2970–2990, 1275, 1020 cm^{-1}.

Anal. Calcd for $C_4H_8Cl_2F_2NOP$: C, 21.26; H, 3.57; N, 6.20. Found: C, 21.39; H, 3.90; N, 6.56.

B.—To 16.86 g (0.11 mole) of $POCl_3$ in 25 ml of dry tetrahydrofuran was added 11.13 g (0.11 mole) of triethylamine in 25 ml of dry tetrahydrofuran and 11.94 g (0.11 mole) of Ib[1] in 25 ml of dry tetrahydrofuran at 0–5°, and the mixture was stored in the refrigerator overnight. The mixture was filtered under nitrogen through a sintered-glass funnel to remove triethylamine hydrochloride. The tetrahydrofuran was evaporated under vacuum and the product was vacuum distilled to give 23.8 g of a colorless liquid, bp 81–83° (0.25–0.20 mm). A small amount of white solid formed in the receiving vessel and this was removed from the colorless product by filtration through a sintered-glass funnel. The liquid was redistilled through a 20-cm, vacuum-jacketed column packed with glass helices. After a forerun, 20.1 g (81%) of IIb was obtained, bp 75–76° (0.25 mm), $n^{25}D$ 1.4593. The nmr and infrared spectra were identical with those of the product obtained from the previous experiment.

Anal. Found: C, 21.40; H, 3.60.

2-[Bis(2-fluoroethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-Oxide (IIIb).—A solution of 6.7 g (0.089 mole) of 3-aminopropanol and 26.5 ml (0.178 mole) of triethylamine in 120 ml of dry dioxane was added with stirring and cooling to 20.12 g (0.089 mole) of IIb in 600 ml of dry dioxane. An exothermic reaction occurred with the formation of insoluble triethylamine hydrochloride, which was filtered after the mixture had been stirred overnight at room temperature. The filtrate was evaporated in a rotary evaporator to give a colorless, nearly clear syrup which was dissolved in 150 ml of dry benzene. The solution was stirred with decolorizing charcoal and filtered, and the filtrate was evaporated in a high-vacuum rotary evaporator at 3×10^{-4} mm to give 10.63 g (52%) of clear, pale yellow syrup: $n^{25}D$ 1.4702; ν_{max}^{NaCl} 3200, 2960–2900, 1225, 1050 cm^{-1}.

Anal. Calcd for $C_7H_{15}F_2N_2O_2P$: C, 36.84; H, 6.63; F, 16.65; N, 12.28. Found: C, 36.81; H, 6.90; F, 15.71; N, 12.14.

A 1.9-g portion of crude product was chromatographed through a Florisil column in benzene. Evaporation in a rotary evaporator gave 0.4 g of clear, colorless syrup, $n^{25}D$ 1.4760.

Anal. Found: C, 36.21; H, 6.54.

N-(2-Chloroethyl)-N-(2-fluoroethyl)phosphoramidic Dichloride (IIc).—A mixture of 18.2 g (0.11 mole) of Ic·HCl[1] and 100 ml (1.1 moles) of $POCl_3$ was stirred and heated at reflux in an oil bath for 18 hr. The resulting light brown solution was concentrated in a rotary evaporator to remove the excess $POCl_3$. The residual oil was filtered through a sintered-glass funnel to remove a small amount of solid, presumably unreacted amine hydrochloride. Vacuum distillation of the filtrate gave a distillate boiling at 100–107° (0.5 mm), which was a mixture of a liquid and solid. The mixture was triturated with dry ether and filtered from about 0.5 g of solid. The filtrate was redistilled through a 10-cm Vigreux column, yielding 20.9 g (77%) of colorless liquid product: bp 97–99° (0.4–0.5 mm); $n^{25}D$ 1.4870; ν_{max}^{NaCl} 2970–2900, 1280, 1020 cm^{-1}.

Anal. Calcd for $C_4H_8Cl_2FNOP$: C, 19.81; H, 3.32; Cl, 43.87; F, 7.84; N, 5.78; P, 12.78. Found: C, 19.51; H, 3.19; Cl, 43.23; F, 7.61; N, 6.02; P, 12.64.

In subsequent preparations in which we attempted to repeat the above synthesis, repeated vacuum distillations through a 10-cm Vigreux column gave fractions which varied widely in refractive indices ($n^{25}D$ 1.4782–1.4877) but which boiled within a 4° range [89.5–93.5° (0.4 mm)]. A small portion of the product was successfully fractionated, however, yielding a fraction, bp 104–106° (1 mm), $n^{25}D$ 1.4860.

Anal. Found: C, 19.94; H, 3.56.

2-[(2-Chloro-2′-fluorodiethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-Oxide (IIIc).—A solution of 3 ml (0.04 mole) of 3-aminopropanol and 15 ml (0.1 mole) of triethylamine in 40 ml of dry dioxane was added to a solution of 9.7 g (0.04 mole) of IIc in 100 ml of dry dioxane. The precipitated triethylamine hydrochloride which formed overnight was removed by suction filtration and the filtrate was evaporated in a rotary evaporator. The remaining oil, containing a small amount of solid, was triturated with dry benzene, treated with charcoal and Celite, and filtered. The filtrate was evaporated to a colorless, viscous oil: 8.7 g (89%); $n^{29}D$ 1.4900; ν_{max}^{NaCl} 3200, 2960–2900, 1225, 1050 cm^{-1}. A portion of the product was chromatographed in benzene on a Florisil column to give a colorless, viscous oil, $n^{25}D$ 1.4907.

Anal. Calcd for $C_7H_{15}ClFN_2O_2P$: C, 34.36; H, 6.18; Cl, 14.49; F, 7.77; N, 11.45; P, 12.66. Found: C, 34.87; H, 6.37; Cl, 14.57; F, 7.82; N, 10.64; P, 12.20.

Additional preparations resulted in crude yields which were nearly quantitative. However, low recoveries were obtained after chromatography (30%). Better analytical results were obtained when the chromatographed product was evacuated at 3×10^{-4} mm pressure in a rotary evaporator at room temperature for 3 hr, $n^{25}D$ 1.4925.

Anal. Found: C, 34.60; H, 6.25; N, 11.10.

Acknowledgment.—We are indebted to Drs. Howard W. Bond, Ronald B. Ross, and Harry B. Wood, Jr., for initiating and stimulating our interest in the fluoro analogs of nitrogen mustard and to Dr. G. Richard Handrick for helpful discussions.

(16) All starting materials and solvents were carefully purified before use The microanalyses were performed by Dr. S. M. Nagy of the Massachusetts Institute of Technology and by the Schwarzkopf Microanalytical Laboratory. The nmr spectra were obtained on a Varian Associates A-60 spectrometer equipped with a V-6040 variable temperature controller and probe. The infrared spectra were obtained with a Perkin-Elmer 237 spectrophotometer.