3-Acetyl-5H-fluoreno[2,3-*d*]oxazolin-2-one (9b).—Compound **9a** (3.00 g, 0.0134 mole) was acetylated by heating it in 30 ml of acetic anhydride for 3 hr. The crude product which had precipitated was recrystallized from dioxane–ethanol to give 3.20 g (90%) of **9b**: mp 263–265° (uncor); ν_{max}^{oil} 1805, 1725 cm^{-1} (C=O).

Anal. Calcd for $C_{16}H_{11}NO_2$: C, 72.44; H, 4.18; N, 5.28. Found: C, 72.53; H, 4.35; N, 5.54.

This product was also obtained directly from the azide (**8d**) in 72% yield by refluxing the latter in acetic anhydride (19 hr). Hydrolysis of **9b** in refluxing 6 *N* ethanolic HCl (19 hr) or in refluxing 1 *N* alcoholic KOH (1 hr) removed only the acetyl group to give **9a**.

Hydrolysis of 9a.—A suspension of 0.90 g (4.0 mmoles) of **9a** in 8.0 ml of 6 *N* HCl containing a few drops of *n*-amyl alcohol as wetting agent was heated in a micro Carius tube at 170° for 4 hr. The pressure was released carefully while cooling the tube in a Dry ice bath, and the solid product was extruded, pressed on a sintered-glass funnel with suction, and dried *in vacuo* over KOH to give 0.95 g (100%) of **2-aminofluoren-3-ol (2d)** hydrochloride, mp 237–240° dec. The free aminofluorenol was obtained in 67% yield by treating the hydrochloride with aqueous Na_2CO_3 and recrystallizing the product from ethanol–water. In other runs, the crude **2d** liberated from the hydrochloride was directly acetylated with acetic anhydride in ethyl acetate–pyridine to **N-(3-acetoxy-2-fluorenyl)acetamide (2g)** in 80% yield, mp 225–226° (lit.[3b] 233–234° uncor), or with acetic anhydride in aqueous Na_2CO_3 to **N-(3-hydroxy-2-fluorenyl)-acetamide (2f)** in 48% yield. In the latter case, work-up of the mother liquor after recrystallization and more vigorous acetylation of the residue derived therefrom afforded an additional 26% as the diacetate **2g**. The infrared spectra of **2d**, **2f**, and **2g** were identical with those of samples prepared previously by different routes.[3b,13a,20]

Ethyl N-(3-Hydroxy-2-fluorenyl)carbamate (2e).—To a cooled, stirred suspension of 2.34 g (10.0 mmoles) of 2-aminofluoren-3-ol hydrochloride prepared according to ref 13a in 50 ml of water was added at once 2.16 g (20.0 mmoles) of ethyl chloroformate and then 50 ml of 0.6 *N* aqueous $NaHCO_3$ dropwise over 5 min. After 1 hr the reaction mixture was diluted with 100 ml of water, and the solid was collected and recrystallized from ethanol (yield 2.33 g, 86%); mp 192–193°; ν_{max}^{KBr} 3420, 3240 (OH, NH), 1700 cm^{-1} (C=O).

Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.16; H, 5.54; N, 5.52.

Potential Carcinolytic Agents.[1] IV. Diazoamino Mustards

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Two new diazoaminofluoro mustards have been synthesized as potential antitumor agents in order to exploit the postulated acidity of the tumor cells. During the attempted preparation of aromatic diazoamino mustards, N,N-bis(2-methanesulfonoxyethyl)-*p*-nitrosoaniline was synthesized and was found to be very effective against a variety of animal tumors.

Various authors[2,3a] of books concerned with the biochemistry of cancer seem to support the idea that the pH of tumor tissues is lower than that of the corresponding normal ones. This is reasonable, since it is known that tumor cells are characterized by a high rate of aerobic and anaerobic glycolysis.[3b] Lactic acid is a dead-end product of glycolysis in tumor cells and, according to Boxer and Devlin,[4] the reduction of pyruvic acid to lactic acid is probably the only "shuttle" available to tumor cells for oxidizing reduced diphosphopyridine nucleotide (DPNH). A bottleneck in the electron transport might cause lactic acid accumulation in tumor cells,[5] especially if the rate of acid production is greater than the combined rates of (a) acid neutralization by buffers diffusing into the tumor cells from the arterial circulation, and (b) acid diffusing out of the cells into the venous blood. The accumulated lactic acid would decrease the pH of tumor cells to a certain limiting value[6] (*ca.* pH 6.0).

Tumor tissues have been shown to be *in vivo* more acidic (pH *ca.* 6.9) than most normal ones (pH *ca.* 7.4), although the evidence is at best circumstantial. The pH was measured with electrodes[7,8] or by determining the quantity of acid-insoluble sulfa drugs precipitated in various tissues.[8–10] (More recently, 5,5-dimethyl-2,4-oxazolidinedione[11] has been used.) Injection of glucose[3c] to the host increases the acidity of the tumor tissues to a pH of *ca.* 6.5. Many animal and human tumors exhibit this behavior although Reichard, *et al.*,[12] found no significant differences in the replacement and recycling of blood glucose in cancer and normal patients (see also ref 13 and 14).

Few investigators have attempted to exploit this physicobiochemical hypothesis as a means of obtaining selective inhibition of the growth of neoplastic cells.

(1) Presented in part at the 151st National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Pittsburgh, Pa., March 28, 1966. Sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-4360. Previous paper: Z. B. Papanastassiou, R. J. Bruni, F. P. Fernandes, and P. L. Levins, *J. Med. Chem.*, **9**, 357 (1966).

(2) J. P. Greenstein, "Biochemistry of Cancer," Academic Press Inc., New York, N. Y., 1954, p 450; A. C. Griffin, "Fundamental Aspects of Normal and Malignant Growth," W. W. Nowinski, Ed., Elsevier Publishing Co., New York, N. Y., 1960; H. Busch, "Biochemistry of the Cancer Cell," Academic Press Inc., New York, N. Y., 1962, p 321.

(3) A. C. Aisenberg, "The Glycolysis and Respiration of Tumors," Academic Press Inc., New York, N. Y., 1961: (a) p 28; (b) p 27; (c) p 1.

(4) G. E. Boxer and T. M. Devlin, *Science*, **134**, 1495 (1961).

(5) S. Weinhouse, *Advan. Cancer Res.*, **3**, 321 (1955).

(6) O. Warburg, K. Posener, and E. Negelein, *Biochem. Z.*, **152**, 309 (1924).

(7) C. Voegtlin, R. H. Fitch, H. Kahler, J. M. Johnson, and J. V. Thompson, *Natl. Inst. Health Bull.*, **No. 164**, 1 (1935); F. F. Beck, R. Musser, C. J. Carr, and J. P. Krantz, *Am. J. Cancer*, **32**, 434 (1938); H. Kahler and W. Robertson, *J. Natl. Cancer Inst.*, **3**, 495 (1943); M. Eden, B. Haines, and H. Kahler, *ibid.*, **16**, 541 (1955); H. Kahler and B. Moore, *ibid.*, **28**, 561 (1962).

(8) J. Naeslund, *Gynecol. Prat.*, **5**, 243 (1954); J.Naes lund and K. Swenson, *Acta Obstet. Gynecol. Scand.*, **32**, 359 (1953).

(9) C. D. Stevens, M. A. Wagner, P. M. Quinlin, and A. M. Kock, *Cancer Res.*, **12**, 634 (1952).

(10) K. A. Meyer, E. M. Kammerling, L. Amtman, M. Koller, and S. J. Hoffman, *ibid.*, **8**, 513 (1948).

(11) D. T. Poole and T. C. Butler, *The Pharmacologist*, **5**, 270 (1963).

(12) G. A. Reichard, N. F. Moury, N. J. Hochella, R. C. Putnam, and S. Weinhouse, *Cancer Res.*, **24**, 71 (1964).

(13) F. Benjamin and S. L. Romney, *Cancer*, **17**, 386 (1964).

(14) D. L. Dewey and F. O. Green, *Biochem. J.*, **72**, 160 (1959).

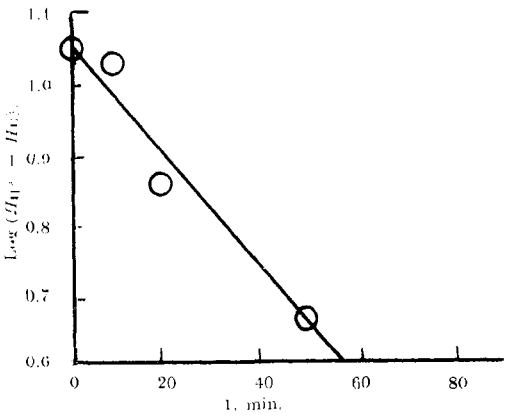


Figure 1. First-order rate plot for formation of II; k = 0.018 min^{-1}, $t_{1/2}$ = 38 min.

In 1961 Ross[15] reported the enhancement of the antitumor activity of basic amino acid nitrogen mustard derivatives in animals pretreated with glucose (see also ref 16), and Kung, *et al.*,[17] reported the glucose potentiation of the antitumor activity of 5-fluorouracil.

In connection with our synthetic program of preparing new deactivated nitrogen mustard derivatives (*i.e.*, agents that do not display cytotoxic activity to normal cells because an electron-withdrawing group, attached to the nitrogen atom of the nor nitrogen mustard moiety, suppresses their ability to alkylate), we have synthesized a number of vinyl mustards which *in vitro* have been shown to release the cytotoxic nor nitrogen mustard moiety when dissolved in slightly acidic media.[18]

We have continued our investigation of deactivated agents to include alkylaryldiazoamino compounds which are known to be rather easily cleaved under slightly acidic conditions.[19]

$$YArN{=}NNR_2 \xrightarrow{H^+} YArN_2^+ + HNR_2$$

If R is 2-chloroethyl and Y is an electron-withdrawing substituent, the compound should be inactive in normal cells but would generate the active cytotoxic agent in the slightly acidic media of the tumor cells. Although such compounds have been reported to undergo a facile intramolecular cyclization,[20] Usbeck, *et al.*,[21] prepared some 8-triazenepurine nitrogen mustards and did not report any cyclization products.

$$YArN{=}NN(CH_2CH_2Cl)_2 \rightarrow \left[\begin{array}{c} YArN{=}{-}N \\ \diagdown\ \ \diagup \\ CH_2CH_2 \end{array} NCH_2CH_2Cl\right]^+ \cdot Cl^-$$

(15) W. C. J. Ross, *Biochem. Pharmacol.*, **8**, 235 (1961).

(16) W. C. J. Ross, *et al.*, *Brit. Empire Cancer Campaign*, **39**, 37 (1961); T. A. Connors, B. C. V. Mitchley, V. M. Roseneer, and W. C. J. Ross, *Biochem. Pharmacol.*, **13**, 395 (1964); T. A. Connors, L. A. Elson, and C. L. Leese, *ibid.*, **13**, 963 (1964).

(17) S. S. Kung, N. D. Goldberg, J. L. Dahl, R. E. Parks, Jr., and B. E. Kline, *Science*, **141**, 627 (1963).

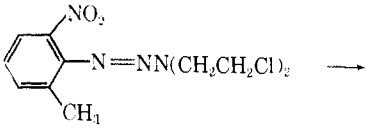
(18) Z. B. Papanastassiou and R. J. Bruni, Abstracts of Papers presented at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1963, p 42L.

(19) N. V. Sidgwick, "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1937, p 457; H. Zollinger, "Azo and Diazo Chemistry," Interscience Publishers, Inc., New York, N. Y., 1961, p 187; K. H. Saunders, "The Aromatic Diazocompounds," Edward Arnold and Co., London, 1947, p 151.

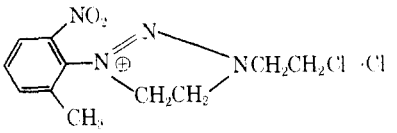
(20) K. A. Kornev and K. K. Khorvenkova, *Ukr. Khim. Zh.*, **25**, 481 (1959).

(21) G. A. Usbeck, J. W. Jones, and R. K. Robins, *J. Am. Chem. Soc.*, **83**, 113 (1961).

Compound I was prepared in the hope that the *ortho* substituents in the benzene ring would hinder the intramolecular cyclization. However, this compound, which was obtained as a crystalline water-insoluble solid, underwent rapid transformation to the water-soluble triazolinium salt II, even in the solid



I



II

state. The study of the transformation was performed either by titrating a sample of the products for chloride ions or, more accurately, by examining the nmr spectra. It was possible to measure quantitatively the relative concentrations of I and II with respect to time by measuring the peak heights due to the methyl groups and thus obtain the rate data presented in Table I. The first-order rate constants for disappearance of I and formation of II (Figure 1) are in excellent agreement. They are probably accurate to within 10%. There was no appreciable difference in rate in the other solvents, *e.g.*, acetonitrile or dimethyl sulfoxide, although nmr absorption by the solvent itself made a quantitative treatment difficult.

TABLE I

RATE DATA FOR THE TRANSFORMATION OF I TO II

t, min	H_I[a]	H_{II}[b]	log (H_I)	log (H_{II}^{∞} − H_{II})[c]
0	18.3	2.4	1.26	1.06
10	12.5	3.7	1.10	1.01
24	8.5	6.9	0.93	0.85
49	6.0	9.3	0.78	0.67
154	1.0	14.0	0.00	
334	0.0	13.7		

[a] Height of methyl peak in I (2.32 ppm). [b] Height of methyl peak in II (2.70 ppm). [c] H_{II}^{∞} = 14.0.

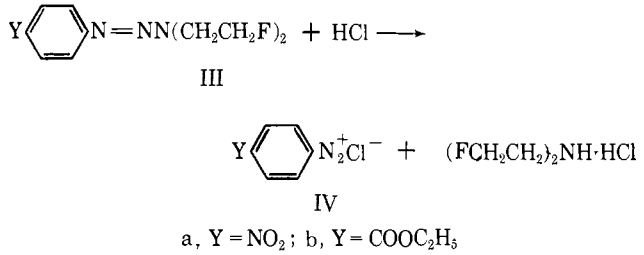
In view of the reported antitumor activity of some bis(2-fluoroethyl)amines[1] and because our nmr studies[22] have shown that 2-fluoroethylamines react through the formation of an aziridinium intermediate like the 2-chloroethylamines but at a slower rate, the diazoaminofluoro mustards III were prepared. After a series of model experiments,[23] optimal conditions were established for the reaction of diazotized *p*-nitroaniline with diethylamine. It was found necessary to remove any undiazotized substituted aniline before carrying out the coupling step; otherwise, the product was contaminated by a symmetrical triazene, $YC_6H_4N{=}NNHC_6H_4Y$. The same conditions were applied for the synthesis of the diazoaminofluoro mustards III. These compounds, in contrast to the corresponding diazoaminochloro mustards I, were stable compounds

(22) P. L. Levins and Z. B. Papanastassiou, *ibid.*, **87**, 826 (1965).

(23) G. R. Rondestredt, Jr., and S. J. Davis, *J. Org. Chem.*, **22**, 200 (1957).

and could be isolated in pure form. The nmr spectrum was unchanged after heating IIIa in acetonitrile at 75° for 15 min or in pyridine at 90° for 1 hr. In acetonitrile solution containing 1 equiv of HCl the expected splitting of the diazoamino moiety was observed by nmr spectroscopy. The reaction could be easily followed by the decrease in intensity of the aromatic peaks due to IIIa and the appearance of a new pair of aromatic doublets due to IVa. Equilibrium was established after *ca.* 11 min, and the resulting spectrum was consistent with reaction shown in Scheme I.

SCHEME I



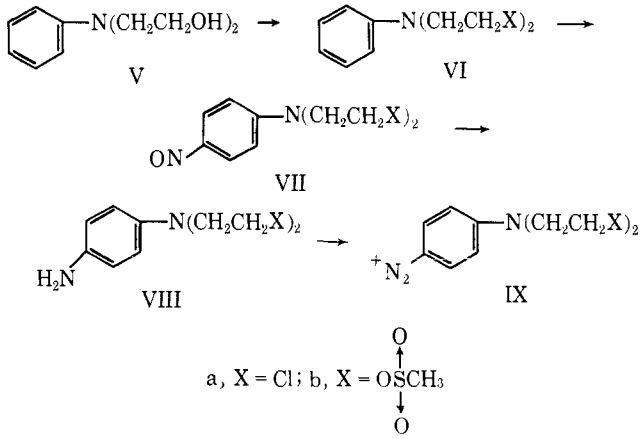
a, Y = NO_2; b, Y = $COOC_2H_5$

Further addition of HCl drove the reaction to completion. Addition of a quantity of 10 *N* NaOH, equivalent to the amount of HCl used, completely restored the original spectrum of IIIa. There was no indication of reaction of IIIa to form triazolinium salts II under the conditions of our experiments.

From the above comparison, it appears that the triazene nitrogens are not as effective in neighboring-group participation in III as in I and in the free amines, 2-fluoro- and 2-chloroethylamine.[22]

Numerous attempts were made to produce some aryl-aryldiazoamino mustards without success. At first we attempted to couple a diazonium salt IV with the phenylenediamine mustards VIII. Only oils were obtained and no pure product could be separated. We attributed this difficulty to the high reactivity of the phenylenediamine mustards VIII. Consequently, the diazonium salts IX were prepared in which the strongly electron-withdrawing diazonium group was expected to stabilize the nitrogen mustard moiety of the molecule (Scheme II). Again, however, our coupling experiments

SCHEME II



with various aniline derivatives yielded tars from which we were unable to isolate any pure substance, although spectral evidence indicated that some of the desired product had formed.

The phenylenediamine mustards VIII were prepared by a method similar to that reported by Ross and Everett.[24] However, in contrast to previous investigators,[25] we were able to obtain the diazonium compounds IX as solid fluoroborate salts.

The elegant method of Cohen and Tipson for preparing VIb was reported[26] after the completion of our experiments.

Antitumor Activity.—Preliminary screening data[27a] show that the fluoro mustards III are devoid of any antitumor activity; IIIa was tested against lymphoid leukemia L1210, Dunning leukemia ascites, Dunning leukemia (ascites, cytoxan resistant), and IIIb against lymphoid leukemia L1210. The inactivity of these fluoro mustards is not surprising because all fluoro analogs of active mustards exhibited minimal activity or were inactive as antitumor agents, *e.g.*, our own work with the fluoro analogs of cyclophosphamide[1] and enamine mustards[28] and that of Martinez, *et al.*,[29] and Dubicki, *et al.*[30] The diazonium fluoroborate salt IXa was active in Walker 256 (subcutaneous) with a therapeutic index of $LD_{10}/ED_{90} \approx (14 \text{ mg/kg})/3 = 5$. The diazonium fluoroborate salt IXb was toxic but prolonged the life of mice implanted with L1210 leukemia to 157% at 1.25 mg/kg, produced 5/6 cures in Dunning leukemia (ascites) at 0.63 mg/kg, and prolonged the life of rats implanted with Dunning leukemia (cytoxan resistant) to 177% at 1.25 mg/kg. The most active compound in this series is the nitroso compound VIIb which is active against 28 out of 37 animal experimental tumors examined, especially in Dunning leukemia (ascites) with a therapeutic index (LD_{10}/ED_{90}) greater than 150, and in intracerebral Dunning leukemia. This compound is currently undergoing preclinical pharmacology and complete results will be published elsewhere.[27b]

The high activity of VIIb in contrast to its precursor VIb which is inactive[26] in the tumors tested could be attributed to a combination of "Myleran" mode of action and nitrogen mustard mode of action.[31] The electron-withdrawing nitroso group reduces the basicity of the amine, and compound VIIb is expected to act by an S_N2 mechanism and be a "Myleran-type" agent. On the other hand, reduction of the nitroso group *in vivo* would produce an active aromatic nitrogen mustard acting by an S_{Ni}-type mechanism. Also, coupling of VIIb with an amine *in vivo* or with a compound containing an active methyl[32] or methylene group could produce an azo compound or a Schiff base, which by themselves would be active aromatic nitrogen mustards. *p*-[Bis(2-methanesulfonoxyethyl)amino]benzaldehyde

(24) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, 1972 (1949); we wish to thank Dr. Ross for a detailed description of the hydrogenation of VIIa to VIIIa; see also W. C. J. Ross, G. P. Warwick, and J. J. Roberts, *ibid.*, 3110 (1955).

(25) W. C. J. Ross and G. P. Warwick, *ibid.*, 1364 (1956).

(26) A. Cohen and R. Stuart Tipson, *J. Med. Chem.*, **6**, 833 (1963).

(27) (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*. We wish to thank Mr. I. Wodinsky, Arthur D. Little, Inc., for permitting us to quote the screening results. For comparison with other compounds, see H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Rept.* **17**, 1 (1962). (b) See I. Wodinsky, Z. B. Papanastassiou, and C. J. Kensler, *Proc. Am. Assoc. Cancer Res.*, **7**, 77 (1966).

(28) Z. B. Papanastassiou and R. J. Bruni, unpublished results.

(29) A. P. Martinez, W. W. Lee, and L. Goodman, *J. Med. Chem.*, **8**, 741 (1965).

(30) H. Dubicki, F. Zielinski, and F. W. Starks, *J. Pharm. Sci.*, **53**, 1422 (1964).

(31) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co. (Publishers) Ltd., London, 1962.

(32) W. Schulze and H. Willitzer, *J. Prakt. Chem.*, **23**, 20 (1964).

was reported to be active against Dunning leukemia.[33] In addition, *p*-nitrosodialkylanilines, even without any biological alkylating moiety, are biologically active (their bacteriocidal activity has been attributed to complexing with nucleic acids).[34]

Experimental Section[35]

3,3-Bis(2-chloroethyl)-1-(2-methyl-6-nitrophenyl)triazene (I) and Its Transformation to 3-(2-Chloroethyl)-1-(2-methyl-6-nitrophenyl)-1,2,3-triazolinium Chloride (II).—A solution of 2.51 g (0.01 mole) of 2-methyl-6-nitrophenyldiazonium hydrogen sulfate (obtained as a light yellow solid, mp 95–105° dec, by diazotizing an alcoholic solution of 2-methyl-6-nitroaniline containing concentrated H_2SO_4 with isoamyl nitrite) in 15 ml of cold water was added dropwise but rapidly to a vigorously stirred solution of 2.76 g (0.0155 mole) of bis(2-chloroethyl)amine hydrochloride and 7.42 g (0.07 mole) of $NaHCO_3$ in 50 ml of water containing a few pieces of ice. The light yellow precipitate was immediately filtered off, pressed dry with filter paper, and placed in a vacuum desiccator over KOH. The nmr spectrum of I was observed within 10 min after preparation: $\delta_{TMS}^{CDCl_3}$ = 2.32 (s), 3.77 (dt), 4.10 (dt), 7.3 (m) ppm.

Titration of a 50% aqueous ethanol solution of the product, immediately after it was pressed dry with filter paper, with 0.1 *N* aqueous $AgNO_3$ solution, using dichlorofluorescein as the indicator, indicated that about 5% of the total chlorine was immediately precipitated as AgCl. Additional chloride ions were slowly released and within 1 hr about 49% of the chlorine content of the sample was precipitated as AgCl. After standing in a vacuum desiccator over KOH overnight, a sample of the product was titrated again. About 26% of the total chloride was immediately precipitated as AgCl and within 15 min the precipitated AgCl amounted to 49% of the total chlorine in the sample.

A sample of this solid (I + II), dissolved in ethanol containing a small quantity of water, was treated with charcoal and precipitated by adding a large excess of ether. A nearly white crystalline material (II) was obtained, which exhibited anomalous melting point characteristics until it was finely ground; a consistent melting point of 80–83° was then obtained; $\delta_{TMS}^{CDCl_3}$ = 2.70 (s), 4.16 (t), 4.89 (t), 5.27 (m), 7.80 (m) ppm.

Anal. Calcd for $C_{11}H_{14}Cl_2N_4O_2 \cdot 1.5H_2O$: C, 39.77; H, 5.16; Cl, 21.35; N, 16.87. Found: C, 39.91; H, 5.48; Cl, 21.53; N, 16.85.

3,3-Bis(2-fluoroethyl)-1-(4-nitrophenyl)triazene (IIIa).—*p*-Nitroaniline (1.38 g, 0.01 mole) was diazotized in 10 ml of concentrated HCl by the addition of 0.7 g (0.01 mole) of $NaNO_2$ in 5 ml of water at 0–5°. After the addition was completed, the mixture was filtered cold and a solution of 1.45 g (0.01 mole) of bis(2-fluoroethyl)amine hydrochloride[36] was added at 0–5° to the filtrate; then 10% NaOH was added until the solution was alkaline. The brownish red solid was collected by suction filtration and dried under vacuum, mp 44–55°. Addition of water to the filtrate produced more crystals, which were collected and dried under vacuum, mp 44.5–46.5°. The two crops were combined (1.0 g) and recrystallized from a solvent mixture of methanol and water to give 0.27 g (10%) of yellow crystals: mp 45–47°; $\delta_{TMS}^{CDCl_3}$ = 4.80 (t), 4.18 (dt), 7.52 (d), 8.25 (d) ppm; $\nu_{max}^{chloroform\ or\ Nujol}$ 2970, 2910, 1605, 1595, 1505, 1035 cm^{-1}.

Anal. Calcd for $C_{10}H_{12}F_2N_4O_2$: C, 46.51; H, 4.68; N, 21.70. Found: C, 46.65; H, 4.90; N, 22.07.

After adding 1 equiv of concentrated HCl to a 0.4 *M* acetonitrile solution of IIIa at 36°, the nmr spectrum exhibited the following peaks: $\delta_{TMS}^{CH_3CN}$ = 8.56 (d), 8.99 (d) ppm (the fluoroethyl protons were resolved but complicated).

3,3-Bis(2-fluoroethyl)-1-(4-carbethoxyphenyl)triazene (IIIb).—The preceeding experiment was repeated using 1.65 g (0.01 mole) of ethyl *p*-aminobenzoate. The yellow solid which precipitated (2.0 g, mp 27–30°), after three recrystallizations from a methanol–water mixture (with charcoal treatment), gave 0.35 g (14%) of triazene as light yellow fluffy needles: mp 32.5–33.5°; $\delta_{TMS}^{CDCl_3}$ = 1.40 (t), 4.40 (q), 4.76 (dt), 4.15 (dt), 7.48 (d), 8.09 (d) ppm; ν_{max}^{neat} 2990–2880, 1715, 1605, 1580, 1505, 1040 cm^{-1}.

Anal. Calcd for $C_{13}H_{17}F_2N_3O_2$: C, 54.73; H, 6.01; N, 14.73. Found: C, 55.00; H, 6.26; N, 15.14.

1,3-Di(4-carbethoxyphenyl)triazene.—Sodium nitrite (0.7 g, 0.01 mole) in 5 ml of water was added at 0 to −5° to a suspension of 3.3 g (0.02 mole) of ethyl *p*-aminobenzoate in 10 ml of 6 *N* HCl. The mixture was made alkaline (pH 9) by the gradual addition of 10% NaOH (20 ml) at 0°. The tan precipitate was collected by suction filtration and recrystallized three times from a methanol–water mixture (with charcoal treatment) to give 0.8 g (24%) of 1,3-di(4-carbethoxyphenyl)triazene: mp 154–155°, lit.[37] mp 152–154°; $\delta_{TMS}^{CDCl_3}$ = 1.40 (t), 4.44 (q), 8.14 (d), 9.51 (s) ppm; $\nu_{max}^{fluorolube}$ 3230, 2980–2880, 1720, 1680, 1605, 1535, 1500 cm^{-1}.

***p*-[Bis(2-chloroethyl)amino]benzenediazonium Fluoroborate (IXa).**—Hydrochloric acid (0.34 ml of 12.2 *N*, 0.0041 mole) was added to a suspension of 1.0 g (0.0037 mole) of N,N-bis(2-chloroethyl)-*p*-phenylenediamine hydrochloride[24] in 12 ml of absolute ethanol at 0°. The mixture was stirred vigorously and 0.60 ml (0.0042 mole) of isoamyl nitrite was added dropwise as rapidly as possible. The mixture was stirred in the cold for 30 min and 2 ml of 48% fluoroboric acid was added dropwise. The sticky black plastic lump gradually broke up into a green powder on further stirring. The green powder was collected by suction filtration and washed twice with two 5-ml portions of absolute ethanol and six times with 25-ml portions of anhydrous ether. Drying in a vacuum desiccator gave 0.79 g (64%) of a light green powder. The product decomposed on heating: $\nu_{max}^{fluorolube}$ 2240 and 2175 cm^{-1}.

Anal. Calcd for $C_{10}H_{12}BCl_2F_4N_3$: C, 36.18; H, 3.65; Cl, 21.36; N, 12.66. Found: C, 35.93; H, 3.87; Cl, 22.01; N, 12.62.

N,N-Bis(2-methanesulfonoxyethyl)-*p*-nitrosoaniline (VIIb).—Methanesulfonyl chloride (138 g, 1.2 moles) in 250 ml of chloroform was added in 2 hr at 0 to −5° to 90 g (0.5 mole) of recrystallized N,N-bis(2-hydroxyethyl)aniline in 500 ml of $CHCl_3$ and 500 ml of pyridine. The solution was allowed to stand for 16 hr at 0° and then washed with six 500-ml portions of 1 *N* HCl and once with water. The solution was dried ($MgSO_4$) and evaporated in a rotary evaporator to a cloudy yellow syrup. The syrup was dissolved in 100 ml of acetone and reprecipitated with 500 ml of ether. The solvent was decanted from the syrup and the solution and reprecipitation were repeated. The remaining solvent was removed in a rotary evaporator under high vacuum leaving 117 g (*ca.* 70%) of a clear, honey-colored syrup, mostly VIb, which was used in the subsequent reaction without further purification: ν_{max} 3500–3400, 3050–2950, 1600, 1510, 1350, 1175 cm^{-1}. The infrared absorption peak at 3400 cm^{-1} indicated the presence of unreacted hydroxyl group; in a subsequent preparation the reaction mixture was allowed to stand at 0° for 5 days and the peak at 3400 cm^{-1} disappeared but the yield was lower.

A solution of 6.7 g (0.097 mole) of $NaNO_2$ in 25 ml of water was added in 20 min at 0–3° to 32.5 g (*ca.* 0.097 mole) of crude N,N-bis(2-methanesulfonoxyethyl)aniline in 80 ml of 6 *N* HCl. The brown tarry mixture was stirred for 1 hr at 5° and then solid Na_2CO_3, about 10 g, was added slowly with stirring until no more CO_2 evolved (pH about 8). The green-black tarry mixture was extracted with three 700-ml portions of CH_2Cl_2 and the combined extracts were cooled at −15° for 70 hr. The dark green crystalline product was collected by suction filtration and dried under vacuum to give 16.7 g of crude product, mp 113–117° dec. Another recrystallization from 700 ml of methylene chloride gave 7 g (20%) of dark green crystals, mp 117–118.5° dec. Thin layer chromatography on Adsorbosil microplates showed: ethyl acetate, R_f 0.60; methanol, R_f 0.70; and ethyl ether, R_f 0.06; $\delta_{TMS}^{CH_3COCH_3}$ = 3.13 (s), 4.10 (t), 4.56 (t), 7.07 (d), 7.83 (d) ppm; $\nu_{max}^{chloroform\ or\ Nujol}$ 3030–2930, 1600, 1525, 1350, 1175 cm^{-1}; $\lambda_{max}^{CHCl_3}$ 395 mμ (ε 2.76 × 10^4), 265 (6.44 × 10^3).

Anal. Calcd for $C_{12}H_{18}N_2O_7S_2$: C, 39.33; H, 4.95; N, 7.65. Found: C, 39.61; H, 5.20; N, 7.55.

***p*-[N,N-Bis(2-methanesulfonoxyethyl)amino]benzenediazonium Fluoroborate (IXb).**—A suspension of 11 g (0.03 mole) of VIIb and 1 g of 10% Pd–C catalyst in 200 ml of ethyl acetate was shaken in a Parr hydrogenation apparatus at an initial hydrogen

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(35) All starting materials and solvents were purified before use. Melting points were obtained with a calibrated Mel-Temp apparatus and are corrected. The infrared spectra were obtained with a Perkin-Elmer 237 spectrophotometer. The nmr spectra were obtained on a Varian Associates A-60 spectrometer equipped with a V-6040 variable-temperature controller and probe (s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet, dt = pair of triplets). The microanalyses were performed by Dr. S. M. Nagy of the Massachusetts Institute of Technology.

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pressure of 2.8 kg/cm² (40 psi). The pressure drop after 90 min indicated that the reduction was 92% complete. Fresh catalyst (0.25 g) was added and shaking under hydrogen was continued for 15 min with no additional hydrogen absorption. The mixture was filtered by suction under nitrogen and the pale yellow filtrate, which started to turn dark immediately, was acidified with dry HCl. The precipitated amine hydrochloride was filtered by suction under nitrogen and immediately dried under vacuum. The green-gray powdery product (9.4 g, 81%) was very hygroscopic and on exposure to air turned instantly to a black tar; it was used in the subsequent preparation without further purification.

Sodium nitrite (1.4 g, 0.02 mole) in 3 ml of water was added dropwise during 10 min to a stirred and cooled (0–5°) solution of 7.8 g (0.02 mole) of crude N,N-bis(2-methanesulfonoxyethyl)-*p*-phenylenediamine hydrochloride in 11 ml of fluoroboric acid (48–50%). The black tarry mixture was stirred an additional 10 min at 5° and the water layer was decanted from the sticky tar. The tar was dissolved in 60 ml of acetonitrile, stirred with decolorizing charcoal, and filtered, and ether was added to the filtrate (20 ml) until crystallization started. The mixture was cooled at −15° for 70 hr and then filtered by suction to give 3.5 g of brownish green crystals, mp 135° dec. The product was recrystallized twice from the same solvent mixture (with charcoal treatment) to give 1.7 g (20%) of dull yellow crystals: mp 135° dec; $\nu_{max}^{(halocarbon\ or\ Nujol)}$ 3030–2950, 2230, 2180, 1590, 1520, 1350, 1175 cm^{-1}.

Anal. Calcd for $C_{12}H_{18}BF_4N_3O_6S_2$: C, 31.94; H, 4.02; N, 9.31. Found: C, 31.43; H, 4.08; N, 9.56.

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Tumor Localizing Agents. Radioactive Iodofluorenaminesulfonic Acids[1]

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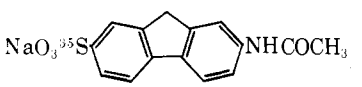
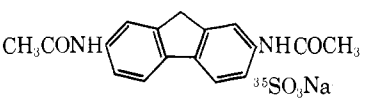
Two compounds, sodium N-2-(3-iodofluorenyl)acetamido-7-sulfonate-^{131}I (VI) and N,N′-2,7-(3-iodofluorenylene)bisacetamido-6-sulfonic-^{131}I acid (XI), were studied for distribution of radioactivity in tumor-bearing mice. The results clearly indicate that both compounds tend to localize in tumor tissue to a greater extent than in many organs or muscle. Compound VI gave the best ratios (concentration in tumor/concentration in tissue) at the end of 8 hr after injection. It showed a statistically significant difference at 0.01 level of probability with liver, kidney, stomach, muscle, and blood, and with spleen at 0.05 level of probability.

For some years, work in these laboratories has been aimed at finding a compound that would display preferential affinity for tumor tissue; if this compound were made radioactive, it could be used in the diagnosis and therapy of internal cancer. Our previous studies have shown that certain ^{35}S-labeled derivatives of fluorenesulfonic acids have an affinity for the tumor tissue.[2] But these compounds did not always give a favorable ratio of uptake of ^{35}S-labeled compound by tumor to other organs, especially liver and kidney. Recently we have elaborated[3] the fluorenesulfonic acid molecule by introducing an additional moiety, the basic amino group, to facilitate the protein binding of the compound.[4] These new fluorenaminesulfonic-^{35}S acids localized in tumor to a greater extent than in vital organs such as kidney, liver, and spleen. One of these compounds, sodium N-2-fluorenylacetamido-7-sulfonate (I), gave a favorable ratio (concentration in tumor/concentration in the tissue) with liver after 16 hr. The favorable ratios with kidney, spleen, and blood increased with increased time. This indicated that the compound is eliminated less readily from the tumor tissue than from the vital organs: it shows the affinity of the substance for the tumor tissue. The second compound, N,N′-2,7-fluorenylenebisacetamido-3-sulfonate-^{35}S (II), gave better ratios of about 4.0 or more at the end of 8 hr with the vital organs. These compounds have shown sufficient relative and absolute tumor tissue concentration to be most suitable for further investigation.



I



II

^{35}S-labeled compounds, while useful for animal experimentation, have the disadvantages associated with low-energy β emission (0.168 mev) for clinical use. It was, therefore, felt that the labeling of these potentially interesting compounds with ^{131}I (a γ and β emitter) might combine ease of detection and estimation with even a possible therapeutic dose of radiation derived from the compound itself since ^{131}I-iodide is used in the therapy of thyroid carcinoma. The use of a γ-emitting isotope could make possible the visualization of tumor tissue by photoscanning. Our attention was focused on the iodination of fluorenaminesulfonic acids because the fluorenamine molecule has been found to retain iodine despite metabolic processes.[5] This should be a great advantage over such compounds as tetrasodium 2-methyl-3-halo-1:4-naphthohydroquinone diphosphate in which the substituted halogen atom at the 3 position was quickly removed from the hydroquinone ring.[6] In the present work, therefore, we

(1) This investigation was supported by U. S. Public Health Service Grant CA 08186 from the National Cancer Institute.

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