After thawing, the volume was measured, and then the urine was filtered through a Seitz filter immediately before determination. This was effected with the twofold dilution method, in broth, using a strain of *Bacillus lateosporus* particularly sensitive to nitrofuran drugs. Excretion was recorded as cumulative per cent of administered substance.

Results

For *in vitro* antibacterial activity, MIC data are reported in Table I. By comparison of the various MIC of I and II, it appears that the activity of the two materials is of the same order of magnitude, although for some bacterial strains II is superior to the polymer.

ED_{50} values for *in vivo* antibacterial activity of I and III are 840 and 345 mg/kg, respectively (confidence limits for 95% probability are 515–1369 mg/kg for I and 272–438 mg/kg for III); this clearly shows a systemic action of the polymer much lower than that of the reference compound.

Urinary excretion of molecular species endowed with antibacterial activity after injections of I and II is shown in Table II. An obvious delay of elimination is observed in the case of the polymer: antibacterial power is observed in urine also at 72 hr, whereas for II it stops at 24 hr.[23] When a similar experiment was tried by oral administration, it showed that I was completely unabsorbed and no antibacterial activity was found in urine.

Acknowledgments.—We thank Professor G. Giacomello for suggestions and help during the execution of this work. We also thank Mr. D. Tricoli for technical aid in the experimental work.

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Fluorene Derivatives for Antitumor Activity[1a]

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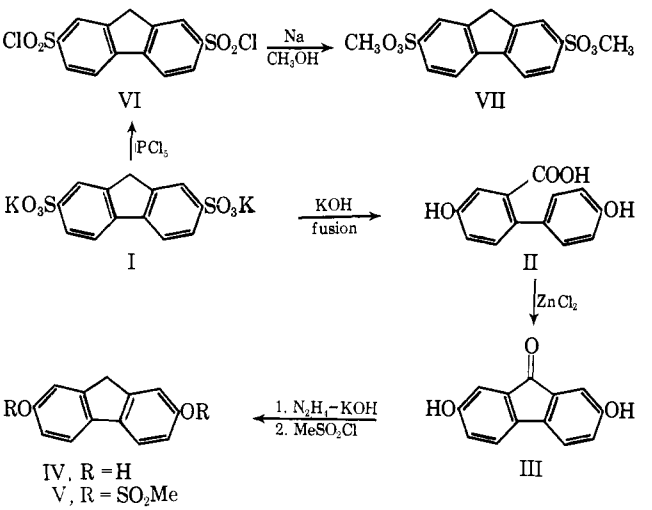
Two compounds 2,7-di(methanesulfonyloxy)fluorene (V) and dimethyl 2,7-fluorenedisulfonate (VII), were prepared for antitumor screening. Methanesulfonyl esters have been reported[2] to be active cancer chemotherapeutic agents. Busulfan, 1,4-di(methanesulfonyloxy)-butane, is a well-known representative of this type of alkylating agent. In order to determine if replacement of the alkane chain of four carbon atoms by a ring system such as fluorene would produce compounds with antineoplastic activity, compound V was made. Compound VII was made to determine the effect of reversing the ester group (S linked to the ring instead of to O).

Compound V was synthesized by the action of methanesulfonyl chloride on 2,7-dihydroxyfluorene (IV) in pyridine solution (Scheme I). The synthesis of IV was attempted by diazotization of 2,7-diaminofluorene followed by hydrolysis; colored condensation products resulted. The synthesis, therefore, was carried out from dipotassium 2,7-fluorenedisulfonate (I), of proved structure.[3] During alkali fusion of I, the ring was broken at C-9 to give 4,4′-dihydroxybiphenyl-2-carboxylic acid (II) which on dehydration with zinc chloride gave 2,7-dihydroxy-9-fluorenone (III).[3] Proof of structure of II was obtained by decarboxylation (heating with lime) to the known 4,4′-dihydroxybiphenyl.[4] Compound III, on reduction by hydrazine hydrate and potassium hydroxide, gave IV.

SCHEME I



Compound VII was prepared from I which was first converted to its dichloride (VI) using phosphorus pentachloride. By the action of sodium and methanol, VI gave the corresponding dimethyl ester (VII).

Biological Results.—Compound VII was administered at a dosage of 50 mg/kg each alternate day to three tumor-bearing, CAF_1/Jax mice. The tumor-curves for these mice are shown in Figure 1A. In the case of two mice there was quite rapid regression of the tumor. The tumor areas of 106 and 128 mm^2 were reduced to 57 and 72 mm^2 on the 9th and 11th day of the administration, respectively, when both the mice died. In the third, the mouse-tumor area of 137 was reduced to 90 mm^2 on the 21st day which was followed by death. The death of all three mice indicated that the toxicity of the compound was high; the dose was reduced to 25 mg/kg. Figure 1B shows the tumor-growth curves on administration of 25 mg/kg of VII. During the time of drug administration the tumor growth seemed to be inhibited. When the drug was stopped, the tumor size increased rapidly, comparable to the controls (Figure 2). After a period of 2 weeks, the tumors of treated mice tended to ulcerate and to expel a core of necrotic tissue, after which, regression of the tumors occurred.

Compound V, at a dosage of 500 mg/kg, also showed inhibited growth patterns (see Figure 3) when compared with controls. These mice also survived with ulceration and expulsion of necrotic tissue. In contrast to these results, two of the control mice died within 21 days due to excessive tumor growth. The third control mouse, however, survived by expelling the core of necrotic tissue. The number of animals in these experiments is too small for conclusive results, but they do in-

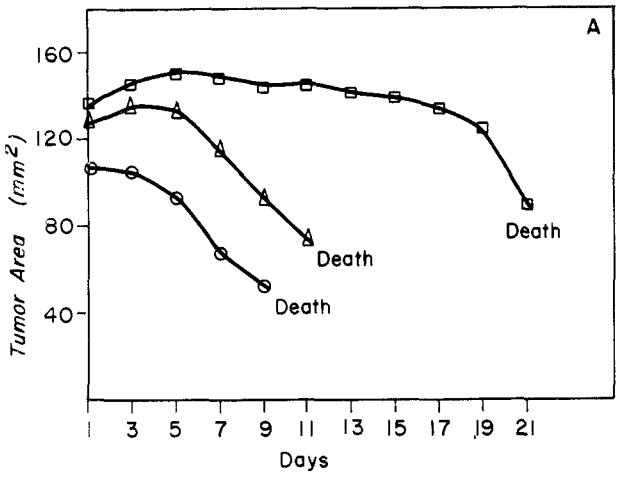
(1) (a) This investigation was supported by Public Health Service Research Grant CA-08186 from the National Cancer Institute. (b) Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut.

(2) T. H. Goodridge, M. T. Flather, R. E. Harmon, and R. P. Bratzel, *Cancer Chemotherapy Rept.*, **9**, 78 (1960).

(3) C. Courtot, *Ann. Chim.*, **14**, 5 (1930).

(4) V. I. Sevastyanov, *Zh. Prikl. Khim.*, **30**, 1858 (1957).

dicate that there is an increase in the life span of treated mice. Weight–response curves of the control and the treated mice are shown in Figure 4. There seems to be no definite effect on the weight of the mice.



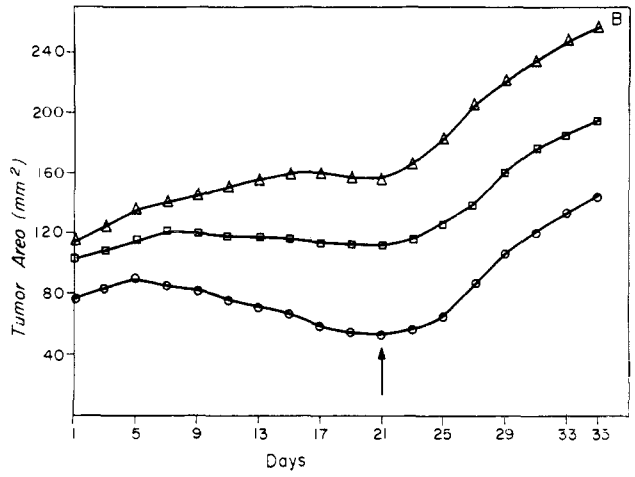


Figure 1.—Growth curves of a transplanted keratinizing squamous cell carcinoma in CAF_1/Jax mice following ip injections of dimethyl 2,7-fluorenedisulfonate (VII) on alternate days. Treatment was stopped after 21 days. A, 50-mg/kg dose; B, 25-mg/kg dose.

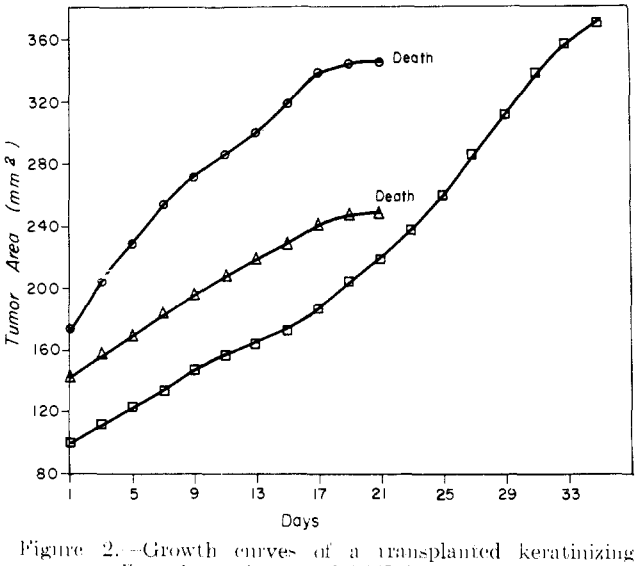


Figure 2.—Growth curves of a transplanted keratinizing squamous cell carcinoma in control CAF_1/Jax mice. The vehicle, 0.5% methylcellulose, was injected intraperitoneally on alternate days.

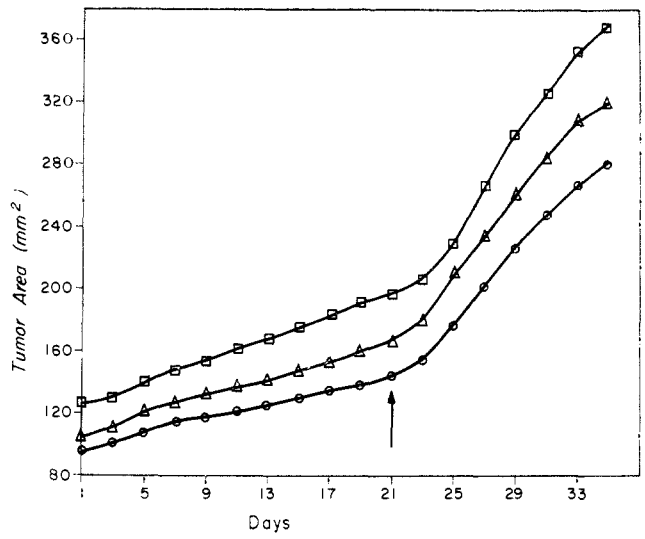


Figure 3.—Growth curves of a transplanted keratinizing squamous cell carcinoma in CAF_1/Jax mice following intraperitoneally injections of 2,7-di(methanesulfonyloxy)fluorene (V) on alternate days. Treatment was stopped after 21 days.

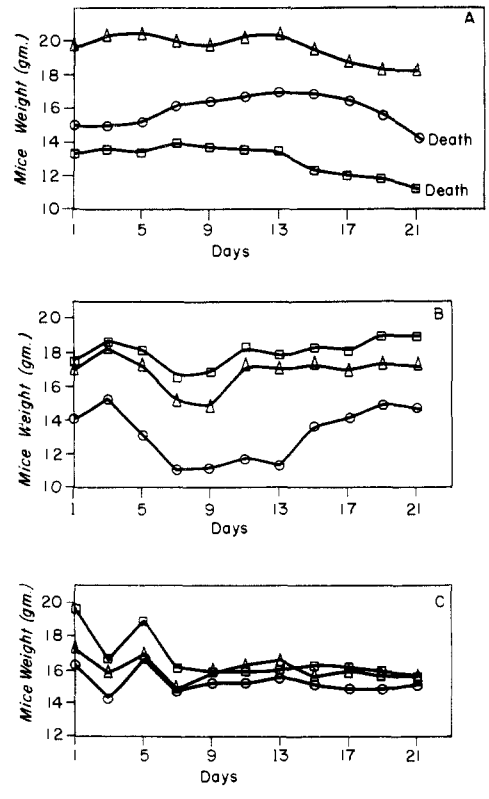


Figure 4.—Weight–response curves. The weights were recorded on alternate days. A, weights for control mice; B, weights for mice treated with dimethyl 2,7-fluorenedisulfonate (VII); C, weights for mice treated with 2,7-di(methanesulfonyloxy)-fluorene (V).

These results indicate that VII had a greater toxicity than V and may have carcinostatic properties in CAF_1/Jax mice. Compound V was relatively nontoxic. The substitution of the fluorene molecule for the alkane (four-carbon) chain of busulfan has reduced activity considerably.

These two compounds were submitted to the Sloan-Kettering Institute for Cancer Research for further screening. The results have revealed no significant inhibition of tumor growth in mouse carcinoma C1025.

Experimental Section[5]

2,7-Dihydroxy-9-fluorenone (III).—4,4′-Dihydroxybiphenyl-2-carboxylic acid[3] (5 g) was pulverized with 10 g of anhydrous $ZnCl_2$. The mixture was heated to 200° for 10 min while stirring constantly, cooled, and decomposed in water. The precipitate was filtered and dried to yield 4.0 g (87%), mp 325–328°. Recrystallization from aqueous alcohol gave dark red crystals, mp 338° (lit.[3] mp 338°).

2,7-Dihydroxyfluorene (IV).—Compound III (2 g) was dissolved in 40 ml of hot diethylene glycol. Hydrazine hydrate (85%, 10 ml) was added, and the mixture was refluxed at 120° for 1 hr. KOH (4 g) was then added, and the temperature was raised to 200–205°. Heating was continued without a condenser for 2 hr. The reaction mixture was then cooled, treated with 30 ml of cold water, and acidified with 12 ml of concentrated HCl to precipitate IV; yield 1.7 g (90.9%), mp 262–263°. Three recrystallizations from aqueous alcohol gave white flakes, mp 269–270°[6] (lit. mp 233°[7] and 249–250°[8]).

Anal. Calcd for $C_{13}H_{10}O_2$: C, 78.78; H, 5.05. Found: C, 78.67; H, 5.12.

2,7-Di(methanesulfonyloxy)fluorene (V).—To compound IV (1 g) in 15 ml of pyridine, 2 ml of methanesulfonyl chloride was added slowly while stirring at 0°. After refrigeration overnight, the contents were poured into 100 ml of cold water. The resulting precipitate was filtered, washed with cold water, and dried. Recrystallization from a mixture of $CHCl_3$ and alcohol gave long, colorless needles, mp 166–167°, yield 1.5 g (84%).

Anal. Calcd for $C_{15}H_{14}O_6S_2$: C, 50.85; H, 3.98; S, 18.08. Found: C, 50.88; H, 3.99; S, 18.04.

2,7-Fluorenedisulfonyl chloride (VI) was made by mixing I (20 g) with PCl_5 (31 g) and heating over a steam bath for 2 hr. The mixture was decomposed in ice, filtered, washed with cold water, and dried under vacuum. It was recrystallized from ethylene dichloride, mp 227° (lit.[9] mp 225°).

Dimethyl 2,7-Fluorenedisulfonate (VII).—To sodium (0.9 g, 20 mmoles) in 100 ml of anhydrous methanol, VI (7.3 g, 10 mmoles) was added in small portions at room temperature. The precipitate was collected, and the filtrate was evaporated under vacuum. The residue was combined with the original precipitate, and the whole was stirred with 25 ml of cold water and filtered. Recrystallization from methanol gave white microneedles, mp 193–194°, yield 4.2 g (60%).

Anal. Calcd for $C_{15}H_{14}O_6S_2$: C, 50.85; H, 3.98; S, 18.08. Found: C, 50.88; H, 4.12; S, 18.29.

Animal Experiments.—Male, CAF_1/Jax mice were employed in this study. Three weeks prior to administration of the compounds, the mice received a subaxially transplant of a keratinizing squamous cell carcinoma.[10] The mice were divided into groups of three. One group consisted of control animals which received 0.2 ml ip of a 0.5% solution of methylcellulose, the vehicle for injecton, each alternate day. Compound V, at doses of 25 and 50 mg/kg and compound VII, at a dose of 500 mg/kg, were administered to three groups of animals. The treatment schedule was continued for 21 days by injecting the compounds on alternate days. On the day of drug administration, estimates of the size of the tumors were made. Tumor areas (mm^2) were plotted against the number of days. Animal weights were also recorded on the same days and plotted. After termination of the treatment, tumor sizes were estimated for another 14 days. The animals were further observed for a period of 5 weeks to determine the number of survivals among treated and control animals.

Acknowledgment.—The author wishes to express his sincere appreciation to Dr. F. E. Ray for helpful discussions and to the Sloan-Kettering Institute for Cancer Research, New York, N. Y., for screening results. Thanks are due the American Cancer Society for an allotment from the University of Florida Institutional Grant IN-62E and 66-7.

(5) Melting points were determined on a Thomas–Hoover capillary apparatus and are recorded as obtained. Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y.

(6) Discrepancies in the melting point of compound IV seems to be due to differences in purity. The purity of compound III is also an important factor in the final purity of IV: lower melting points resulted from the use of crude III.

(7) C. D. Nenitzescu and M. Avram, *Acad. Rep. Populare Romine, Studii Cercetari Chim.*, **4**, 57 (1956).

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(10) Line a, stomach carcinoma originally obtained from the animal supply and research units of the British Empire Cancer Campaign.

New Alkylating Agents Derived from Diaziridine[1]

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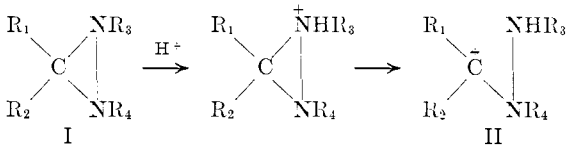
Several of the best known biological alkylating agents that were found effective as antineoplastic drugs[3] and/or insect chemosterilants[4] contain aziridine (ethylenimine) rings as their reactive functional groups. In this laboratory there has been particular interest in the synthesis and study of a series of ring-substituted bis(1-aziridinyl)phosphinylurethans ("dual antagonists")[5] as well as such other ring-substituted aziridine derivatives[6] that might be capable of undergoing SN1-type reactions under biological conditions.[7]

Diaziridines (I) were shown recently to undergo acid hydrolysis by an SN1 mechanism[8] which must involve ring opening with the formation of a carbonium ion (II). It was thought that diaziridines might act as



biological alkylating agents if their ring-opening reaction is sufficiently "activated" by electron-attracting R_3 substituents similar to those that were proved effective in the case of the aziridines (C=O, P→O, P→S, etc.). It seemed, therefore, of interest to prepare some diaziridinyl analogs of the ring-substituted bis(1-aziridinyl)phosphinylurethan[5] and tris(1-aziridinyl)phosphine oxide[3] series of known chemotherapeutic agents.

While on the basis of analogy[6] and rationale,[7] the corresponding derivatives of 3,3-dialkyldiaziridines would seem to be the most desirable ones; unfortunately, such diaziridine rings (derived from ketones) are known to be unstable toward acylating reagents.[9,10]

(1) This investigation was supported by Grant CA-06695 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

(2) To whom inquiries should be directed.

(3) See, *e.g.*, D. A. Karnofsky and F. Bergel in "Chemotherapy of Cancer," P. L. Plattner, Ed., Elsevier Publishing Co., New York, N. Y., 1964, pp 3–18 and 21–31.

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