and the purple mixture became dark brown. The reaction was stirred at room temperature for several hours. Cracked ice was added, and the mixture was stirred until the excess acetic anhydride hydrolyzed. The resulting solid was collected by filtration and washed with water to give 888 mg of a brown solid. This solid was washed with cold methanol to give 467 mg of a yellow solid, mp 195–200°. This material was used in the next step without further purification.

A stirred mixture of 2.053 g (5 mmoles) of the above solid in 107 ml of water and 15.7 ml of 25% NaOH was heated at reflux under nitrogen until solution occurred. The solution was filtered and a stream of air was introduced into the filtrate for 40 min. The resulting purple solution was acidified by addition of HCl. The resulting orange solution was extracted with CH_2Cl_2 and the extracts were washed with water. The dried extracts were evaporated, and the residue was recrystallized from CH_2Cl_2–petroleum ether to give 410 mg (33%) of red crytals: mp 172–175°; λ_{max} 227, 298, 338 mμ (ε 19,050, 13,700, 4950); λ 3.01, 5.99, 6.05, 6.21, 8.9 μ.

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 63.08; H, 5.58; N, 5.43.

3-Acetyl-1-ethyl-5-methoxy-2,6-dimethylindole-4,7-dione.—3-Acetyl-1-ethyl-5-hydroxy-2,6-dimethylindole-4,7-dione (163 mg, 0.625 mmole) was alkylated with 4 ml of methyl sulfate in the presence of 1.6 mg of K_2CO_3 as described for IIa. The product (91 mg, 53%) was obtained from CH_2Cl_2–petroleum ether as orange crystals: mp 126–127°; λ_{max} 222, 286, 338 mμ (ε 20,200, 11,400, 3990); λ 5.95, 6.01, 6.07, 6.17, 8.98 μ; pmr, 80 (3t, J = 7.0 cps, CH_2CH_3), 118 (3s, 6-CH_3), 144 (3s, $COCH_3$), 159 (3s, 2-CH_3), 241 (3s, CH_3O), 265 cps (2q, J = 7.0 cps CH_2CH_3).

Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.77; H, 6.51; N, 5.35.

1-Ethyl-3-(1-hydroxyethyl)-5-methoxy-2,6-dimethylindole-4,7-dione.—A stirred solution of 100 mg (0.3 mmole) of 3-acetyl-1-ethyl-5-methoxy-2,6-dimethylindole-4,7-dione in 10 ml of methanol was heated to reflux and 100 mg of $NaBH_4$ was added under nitrogen. The resulting mixture was heated for 1–2 min and then allowed to stir for 1 hr at room temperature. To the solution was added 1 ml of acetone, and after 5 min 1 ml of 1 N $FeCl_3$ in 0.1 N HCl solution. The resulting mixture was distributed between CH_2Cl_2 and water. The organic phase was washed with saline, dried, and evaporated to give a reddish oil having λ_{max}^{MeOH} 230, 287, 358 mμ, and λ_{max}^{KBr} 2.90, 6.05, 6.16, 6.20, 9.10, 10.00 μ. This oil was used in the next step without further purification.

1-Ethyl-3-(1-hydroxyethyl)-5-methoxy-2,6-dimethylindole-4,7-dione Phenylcarbonate.—To an ice-chilled, stirred solution of 300 mg (1.08 mmoles) of crude 1-ethyl-3-(1-hydroxyethyl)-5-methoxy-2,6-dimethylindole-4,7-dione in 6 ml of pyridine was added 0.3 ml of phenyl chloroformate. The resulting mixture was stirred and warmed intermittently on the steam bath over a 3-hr period. The mixture was poured into water and extracted with CH_2Cl_2. The combined extracts were washed with saline, dried, and evaporated to give 290 mg of an orange oil which was used for the subsequent step without purification.

1-Ethyl-3-(1-hydroxyethyl)-5-methoxy-2,6-dimethylindole-4,7-dione Carbamate (Ib).—Ammonia gas was introduced into a solution of 90 mg of crude 1-ethyl-3-(1-hydroxyethyl)-5-methoxy-2,6-dimethylindole-4,7-dione phenylcarbonate in 20 ml of ether, chilled in a Dry Ice bath, until an equal volume of NH_3 had condensed. The Dry Ice bath was removed, and reaction was allowed to stand under a Dry Ice condenser for 4 hr. The excess NH_3 was allowed to evaporate, and the reaction was diluted with ether, washed with saline, dried, and evaporated to give an orange oil. The oil was chromatographed on Celite (diatomaceous silica) using a heptane–ethyl acetate–methanol–water (70:30:17:4) system[8] the fraction with peak hold-back volume 2.94 (V_m/V_s = 2.34) was evaporated to give 15 mg of an orange oil: λ_{max} 232, 285, 348, 460 mμ (ε 14,350, 11,650, 2720, 1120); λ_{max} 2.82, 2.9, 5.77, 5.99, 6.06 μ; pmr, 79 (3t, J = 7.5 cps, CH_2CH_3), 94 (3d, J = 7.0 cps, CH_3CHO), 116 (3s, 6-CH_3), 141 (3s, 2-CH_3), 239 (3s, CH_3O), 259 (2q, J = 7.5 cps, CH_2CH_3), 294 (2 broad, NH_2), 373 cps (1 q, J = 7.0 cps, CH_3CHO).

3-Chloromethyl-1-ethyl-5-methoxy-2,6-dimethylindole-4,7-dione (IX).—A solution of 100 mg (0.38 mmole) of 1-ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione (VIII)[2] and 5 ml of $SOCl_2$ was stirred at room temperature for 40 min. The excess $SOCl_2$ was evaporated, and the product was isolated with CH_2Cl_2. The residue was recrystallized from ether–petroleum ether to give 75 mg (70%) of yellow crystals: mp 141–142°; λ_{max} 232, 285, 348, 455 mμ (ε 17,050, 14,050, 5630, 1270); λ 6.03, 6.09, 6.2 μ.

Anal. Calcd for $C_{14}H_{16}ClNO_3$: C, 59.68; H, 5.72; N, 4.97; Cl, 12.58. Found: C, 59.79; H, 5.87; N, 4.45; Cl, 12.56.

3-Acetylthiomethyl-1-ethyl-5-methoxy-2,6-dimethylindole-4,7-dione (X).—A solution of 50 mg (0.17 mmole) of 3-chloromethyl-1-ethyl-5-methoxy-2,6-dimethylindole-4,7-dione (IX) and 20.3 mg (0.17 mmole) of potassium thioacetate in 10 ml of acetone was stirred at room temperature for 1 hr. The solution was diluted with water to give 46.7 mg (81%) of solid, mp 109–111°. A sample recrystallized from CH_2Cl_2–petroleum ether had mp 111–112°; λ_{max} 230, 286, 355, 465 mμ (ε 21,000, 13,650, 3210, 1670); λ 5.95, 6.10, 6.25 μ.

Anal. Calcd for $C_{16}H_{19}NO_4S$: C, 59.80; H, 5.96; N, 4.36; S, 9.97. Found: C, 59.47; H, 6.12; N, 4.28; S, 9.87.

1-Ethyl-5-methoxy-3-methoxymethyl-2,6-dimethylindole-4,7-dione (XII).—A solution of 100 mg (0.38 mmole) of 1-ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione phenylcarbonate (XI)[2] in 10 ml of methanol was heated on the steam bath for 10 min. The solvent was evaporated, and the residue was recrystallized from dilute methanol to give red crystals: mp 90–91°; λ_{max} 231, 285, 345, 462 mμ (ε 15,900, 12,700, 3040, 1105); λ 6.01, 6.13, 6.25, 9.02, 9.24 μ; pmr, 79.5 (3t, J = 7.0 cps, CH_2CH_3), 117 (3s, 6-CH_3), 137.5 (3s, 2-CH_3), 204 (3s, CH_2OCH_3), 239.5 (3s, 5-CH_3O), 260 (2q, J = 7.0 cps, CH_2CH_3), 276 cps (2s, CH_2OCH_3).

Anal. Calcd for $C_{15}H_{19}NO_4$. C, 64.96; H, 6.91; N, 5.05. Found: C, 65.51; H, 7.20; N, 5.07.

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A Polymeric Nitrofuran Derivative with Prolonged Antibacterial Action[1a]

FRANCA ASCOLI, GIOVANNI CASINI,[1b] MARCELLO FERAPPI, AND EZIO TUBARO

Istituto di Chimica Farmaceutica e Tossicologica dell'Università di Roma, Rome, Italy

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Recently some attempts have been reported to obtain polymers possessing pharmacological activity. Besides studies involving the preparation of polymeric models of natural macromolecules (with more or less known pharmacological activity), polymers have been sought, whose monomeric units were endowed *per se* with some pharmacological activity, because of the groupings present in them. The general aim was to obtain macromolecules which would, at least partially, retain the activity of the monomer. A slow liberation of monomeric units *in vivo* might lead to a prolongation of action, and thereby attain drug latentiation.[2]

Such macromolecular drugs may, of course, show inherent new physiological activities and a characteristic toxicity of their own. This has been noticed for some specific polymers which induce renal lesions.[3] A

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TABLE I
MINIMUM INHIBITORY CONCENTRATION OF 5-NITRO-2-FURALDEHYDE POLYACRYLOYLHYDRAZONE (I) AND 1-[(5-NITROFURFURYLIDENE)AMINO]HYDANTOIN (II)

Microorganism	MIC, μg/ml[a] I	II
Staphylococcus aureus	25	12.5
Streptococcus β-hemolyticus	1.56	0.19
Escherichia coli	12.5	6.25
Shigella flexneri	12.5	3.12
Proteus vulgaris	12.5	12.5
Sarcina lutea	12.5	12.5
Mycobacterium phlei	100	100
Salmonella typhosa	12.5	12.5
Bacillus cereus	25	12.5
Klebsiella pneumoniae	12.5	12.5
Bacillus subtilis	25	12.5
Alcaligenes faecalis	12.5	12.5
Pseudomonas aeruginosa	12.5	12.5

[a] 24-hr incubation.

disease, named "macromolecular hypertension," was described; it was supposed to be caused by a blockade of the glomerular ultrafiltration.[4] Nevertheless many synthetic polymers are nontoxic, *i.e.*, polyvinylpyrrolidone (PVP), which is used as a plasma substitute.

Attempts to obtain polymers with pharmacological activity have been applied mainly to antibacterial monomers. For instance, a urea–formaldehyde polycondensate has been described, which possessed antibacterial activity not exclusively due to a slow liberation of formaldehyde *in vitro*.[5] *p*-Aminosalicylic acid (PAS) has been bound to the polyaldehyde obtained by oxidation of starch, giving a tasteless derivative which was better tolerated than PAS, although it was still active and possesses prolonged action.[6] A group of Russian workers has further developed this concept, mainly by introducing antibacterial units such as PAS or penicillin into macromolecules[7–11] (for a review see ref 12). More recently the preparation and the antibacterial activity of a polymeric tropolone derivative was reported,[13,14] and a sulfapyridine–formaldehyde copolymer was described, possessing antimalarial activity greater than the parent sulfonamide drug.[15]

In our laboratory, initial results have been obtained with the preparation of a polymeric nitrofuran model, 5-nitro-2-furaldehyde polyacryloylhydrazone (I), by condensing polyacryloylhydrazide with 5-nitro-2-furaldehyde. This material shows antibacterial activity *in vitro* (Table I) similar to that of a classic nitrofuran drug, 1-[(5-nitrofurfurylidene)amino]hydantoin (II).[16] Although the compound, like most other nitrofuran drugs, is practically devoid of systemic action *in vivo*, it is interesting to note that the antibacterial activity found in the urine after a single parenteral dosage, is much longer lasting than that observable after II, and can still be demonstrated 72 hr after administration (Table II). The substance is completely unabsorbed by the oral route, so that a practical usefulness of the polymer prepared by us is unlikely. Our findings have, however, shown the possibility of inducing latentiation in the nitrofuran drugs.

TABLE II
URINARY EXCRETION[a] OF 5-NITRO-2-FURALDEHYDE POLYACRYLOYLHYDRAZONE (I) AND 1-[(5-NITROFURFURYLIDENE)AMINO]HYDANTOIN (II)

Lot	I, % recovered 24 hr	18 hr	72 hr	II, % recovered 24 hr
1	12.8	26.1	35.4	44.4
2	11.5	22.0	28.6	31.8
3	14.1	25.2	35.6	25.6
4	9.9	22.7	31.3	37.9
Av	12.1	24.0	32.7	34.9

[a] After 100 mg/kg im. Urinary levels were biologically measured and averaged on five animals.

Experimental Section

5-Nitro-2-furaldehyde Polyacryloylhydrazone (I).—Polyacryloylhydrazide (2 g, 0.023 mole) was dissolved in water (30 ml) and added to a solution of 5-nitro-2-furaldehyde (4.95 g, 0.035 mole) in 50% aqueous ethanol (100 ml) containing acetic acid (15 ml). Immediate precipitation of a yellow solid was observed; the reaction flask was shaken occasionally and, after 1 hr, the yellow solid was collected on a filter and washed with small amounts of water. The product was purified by dissolving in dimethylformamide (DMF) and reprecipitating by adding water containing little methanol. The treatment was repeated twice and the purified product was vacuum dried at 100°, mp 230° dec, λ_{max}^{DMF} 372 mμ (log ε 4.089). The product was soluble in DMF, slightly soluble in chloroform, xylene, acetone, dioxane, and ethyl acetate, and insoluble in cyclohexane, petroleum ether (bp 30–70°), methanol, ethanol, and water.

Anal. Calcd for $(C_8H_7N_3O_4)_n$: C, 45.94; H, 3.37; N, 20.09. Found: C, 45.61; H, 3.83; N, 19.75.

The polyacryloylhydrazide used as starting material was prepared following Kern and co-workers[17] from methyl polyacrylate and hydrazine; it showed η_{sp} 0.26 (*c* 0.1, water).

In Vitro **Antibacterial Activity.**—Minimum inhibitory concentration (MIC) measurements were effected by the twofold dilution method according to Braude and Dockrill.[18]

In Vivo **Antibacterial Activity.**—Experimental infection was effected following Sackmann and Neipp,[19] using a culture broth of *Staphylococcus aureus* (Smith ATCC 13709) containing 1% (w/v) of sodium glycocolate and taurocholate.[20] Protection was effected 1 hr after infection, injecting subcutaneously suspensions of I and of 5-morpholinomethyl-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone (III) (a systemic nitrofuran)[21] at dosages of 0.500, 0.250, and 0.125 g/kg, in lots of 12 mice each (17–20 g). Suspensions were prepared from the micronized powder (150 mesh) in water containing Tween 80. ED_{50} values were calculated by the method of Litchfield and Wilcoxon.[22]

Urinary Excretion.—Male albino Wistar rats (200–250 g) were divided in lots of five animals each, fasted for 24 hr and then injected with 0.100 g/kg of I and II. For both groups of determinations the same animals were used, after a 15-day rest. Urine was collected in Dry Ice refrigerated erlenmeyer flasks.

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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.

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Fluorene Derivatives for Antitumor Activity[1a]

KRISHNA C. AGRAWAL[1b]

Pharmaceutical Chemistry Research Laboratory, University of Florida, Gainesville, Florida

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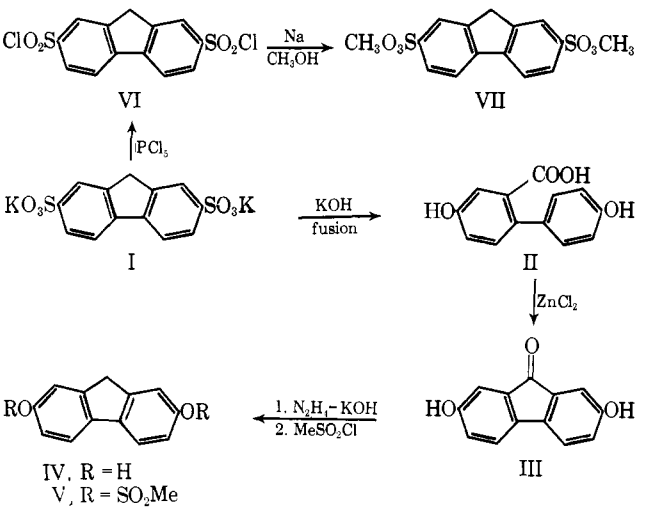
Two compounds 2,7-di(methanesulfonyloxy)fluorene (V) and dimethyl 2,7-fluorenedisulfonate (VII), were prepared for antitumor screening. Methanesulfonyl esters have been reported[2] to be active cancer chemotherapeutic agents. Busulfan, 1,4-di(methanesulfonyloxy)-butane, is a well-known representative 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-

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