1.5 g (57%) of the hydrochloride of the desired product (8) which melted at 202–204° (2-butanone–MeOH).

Triarylhaloethylenes. Method A. 2-[*p*-[2-(*p*-*tert*-Butylphenyl)-2-chloro-1-phenylvinyl]phenoxy]triethylamine Dihydrogen Citrate (2). A 10.0-g (0.0216 mol) quantity of the hydrochloride of 2-[*p*-[2-(*p*-*tert*-butylphenyl)-1-phenylvinyl]phenoxy]triethylamine[4] in 30 ml of $CHCl_3$ was stirred and treated dropwise with 37.9 ml of a 0.57 *M* solution of Cl_2 in CCl_4 (0.0216 mol). The temperature was maintained below 40° during the addition. The solution was stirred for 0.5 hr after the addition of Cl_2 and then it was heated under reflux for 1.5 hr. It was cooled to room temperature and neutralized with aqueous $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, and filtered. The filtrate was treated with 4.2 g (0.216 mol) of citric acid in 10 ml of MeOH and the solvents were evaporated. The residue was recrystallized first from 2-butanone and then from EtOH–Et_2O to give 7.6 g (54%) of product melting at 117–119°.

Triarylhaloethylenes. Method B. *N*-[2-[*p*-(2-Chloro-1,2-diphenylvinyl)phenoxy]ethyl]pyrrolidine (3). A mixture of 9.2 g (0.03 mol) of *p*-(2-chloro-1,2-diphenylvinyl)phenol, 5.1 g (0.03 mol) of pyrrolidinoethyl chloride hydrochloride, 4.0 g (0.10 mol) of NaOH, 100 ml of H_2O, and 100 ml of benzene was heated under reflux for 4 hr. The organic layer was then separated, dried over $MgSO_4$, filtered, and evaporated. The residue was taken up in ether, percolated through alumina, and treated with 5.9 g (0.03 mol) of citric acid in 2-butanone. The solid which separated was recrystallized twice from 2-butanone to give 9.2 g (51%) of product which melted at 121–125°.

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Polymeric Anions and Biological Activities. Effects on Intramuscular and Intraperitoneal Walker Carcinosarcoma 256 of the Rat

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Inhibition of growth of several murine tumors by some water-soluble polymers has been demonstrated by Regelson.[1] Polyanions also possess other important biological properties, such as the ability to inhibit the growth of viruses, to induce the production of interferon *in vivo*, and to increase the immune response[2] which may be related to their inhibition of tumor growth. The purpose of this work is to elucidate the structure–antitumor relationship for a variety of polyanions for both the solid (intramuscular) and the ascitic (intraperitoneal) forms of the Walker carcinosarcoma 256 of the rat.

The antitumor activities of these materials were determined by the National Cancer Institute by the protocols which have been established by them[3] against intramuscular and intraperitoneal Walker carcinosarcoma 256 of rats. The results are shown in Tables I and II.

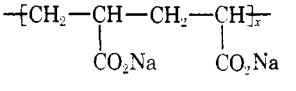
Toxicity and Structure. Since these polymers were administered at the maximum tolerated level, the largest doses used (as shown in Tables I and II) are some indication of their toxicities. For a given series of compounds

Table I. Effectiveness against the Intramuscular Walker Carcinosarcoma 256 of the Rat

Polymer	Av[a] mol wt	Dose,[b] mg/kg	*T/C*,[c] %
Poly(ammonium acrylate) (A)	50,000	400	127
Poly(ammonium acrylate) (B)	100,000	400	94
Poly(sodium acrylate) (A)	250,000	400	41
Poly(sodium acrylate) (B)	10,000,000	400	31
		200	73
		100	67
		50	84
Poly(sodium acrylate) (C)	10,000,000	20	21
		10	25
		5	46
		2.5	89
Poly(sodium acrylate) (D)	10,000,000	22	24
		11	30
		5.5	46
		2.7	92
Ethylene–maleic anhydride copolymer (A)	4,100	400	83
Ethylene–maleic anhydride copolymer (B)	17,000	15	26
		7	50
Ethylene–maleic anhydride copolymer (C)	43,000	10	40
		5	54
		2.5	73
Ethylene–maleic acid copolymer	17,000	200	52
Acrylic acid–acrylamide copolymer	5,000,000	200	70
Acrolein, polymer with $NaHSO_3$	100,000	400	83

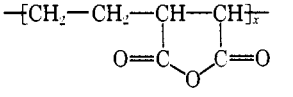
[a] These compounds have a range of molecular weights that is usual for most unfractionated polymers. [b] Four ip injections were given to six rats at 24-hr intervals, starting on the third day after the solid tumor was introduced beneath the skin of the rat. [c] Animals were sacrificed on day 7 and the tumors of the treated (T) and control (C) animals were weighed. *T/C* should be 53% or less for statistically significant antitumor activity, and four of the six rats must be alive on day 5 for a valid test.[3]

toxicity increases as the molecular weight increases. Of the three copolymers of ethylene and maleic anhydride listed, the one with the lowest molecular weight could be used at the level of 400 mg/kg of rat, but the copolymer with the highest molecular weight of this series could be used at no more than 10 mg/kg without lethal effects. Other factors including the ionic charge must also affect the toxicity since poly(sodium acrylate) (structure a),



a

with a molecular weight of 250,000, could be used safely at 400 mg/kg, whereas poly(ethylene-maleic anhydride) (structure b), with a molecular weight of 43,000, could be used safely at no more than 10 mg/kg.



b

It is interesting to note that Breslow, *et al.*,[4] found that different molecular weight cuts of the copolymer of divinyl ether and maleic anhydride have relatively different

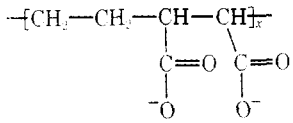
Table II. Effectiveness against the Intraperitoneal Walker Carcinosarcoma 256 of the Rat

Polymer	Av[a] mol wt	Dose,[b] mg/kg	T/C,[c] %
Poly(sodium acrylate) (C)	10,000,000	100	110
		50	131
		25	164
		12.5	242
		4.5	148
		2.3	168
		1.1	158
		0.3	188
Poly(sodium acrylate) (D)	10,000,000	160	174
		80	174
		40	156
		20	169
		10	182
		5	169
		3.5	167
		1.7	162

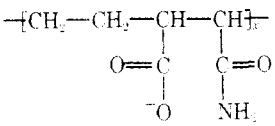
[a] These compounds have a range of molecular weights that is usual for most unfractionated polymers. [b] Nine ip injections were given to six rats at 24-hr intervals, starting on the third day after the ascities tumor cells were introduced. [c] Survival times of the treated (T) and the control (C) rats were determined. T/C should be 125% or more for statistically significant antitumor activity, and four of the six rats must be alive on day 5 for a valid test.[3]

toxicities and therapeutic effects, indicating some advantage in the use of fractions of the polymer with smaller ranges of molecular weights.

Activity against the Intramuscular Walker Carcinosarcoma 256 of the Rat. The activities of these polymers against the Walker carcinosarcoma 256 of the rat increase as the ionic charges on the molecule increase in number. The copolymer of ethylene and maleic anhydride with a molecular weight of 17,000 has a T/C value of 26% at a dose level of 15 mg/kg and exists in neutral solution as



The copolymer of ethylene and maleamic acid with a molecular weight of 17,000 has a T/C value of 52% at a dose level of 200 mg/kg and exists in neutral solution as



The latter at dose levels 13 times as great as the former has only one-half the activity against this tumor.

The activities of these polymers increase as the molecular weight increases, but unfortunately the toxicities increase also. Although the effective dose is smaller for the compounds with greater molecular weights, the maximum T/C values do not vary with an increase in molecular weight after a minimum molecular weight is attained.

Activity against the Intraperitoneal Walker Carcinosarcoma 256 of the Rat. The two polymers tested in this tumor system (Table II) were similar in their behavior. Each caused little change in survival times as the dose levels decreased from 40-50 to 0.3-1.7 mg/kg, a 100-fold change in dose levels. The activity at these low doses indicates an effect on the cells which slows their growth but does not kill them.

Discussion of Results

One injection of the polymer at the level of 1 mg/kg of rat contains 10^{13} molecules of the polymer if one assumes a 220-g rat and a molecular weight of 10,000,000. Since the original injection contains approximately 10^6 cells, there are about 10^7 molecules of polymer per tumor cell inoculated. If these polyanions were acting directly on the surface of the cancer cells one would expect them to be more effective against the ascitic cells which are in direct contact with the compounds than against solid tumors which require transport of the polymers to the active site. However, their activities against the solid tumor appear to be greater than their activities against the ascitic cells.

An interesting speculation concerning this difference in activity is aroused by a paper by Elias and Brugarolas[5] who used heparin in combination with other drugs in the treatment of four patients with solid tumors. Concomitant use of heparin caused improvement in the patients who had failed to respond to the same drugs without the heparin. Since these polymers are similar to heparin in being high molecular weight, highly charged polyanions, they may have heparin-like properties, including anticoagulant effects. Shamach and Alexander have shown that some synthetic polyanions do prevent clotting by inhibiting the conversion of fibrinogen to fibrin.[6]

The antitumor effects of these synthetic polyanions on solid tumors may be the result of indirect effects such as an increase in the immune response. Regelson and others[7] have demonstrated host-mediated responses caused by synthetic polyanions.

Experimental Section

Since these polymers have been known[8] for some time, their syntheses are not described here. The weight-average molecular weights of the materials used were checked by means of the viscosity of aqueous solutions buffered to pH 7.0.[9]

Acknowledgment. Grateful acknowledgment is made of the valuable assistance of Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, for providing the antitumor screening data on these compounds. Dr. William Regelson very kindly supplied a preprint of the last paper listed in ref 2.

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Antiinflammatory Sydnones. 1

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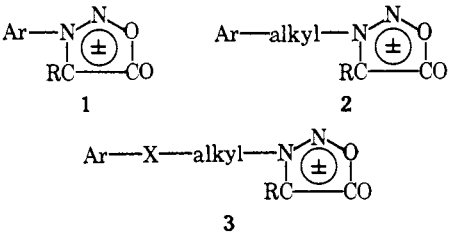
Mesoionic compounds have been studied extensively as possible medicinal agents but, while many have proved interesting, none has shown activity at levels sufficiently high to be of practical interest.[1]

In an attempt to develop a series of mesoionic compounds with consistent biological activity, the synthesis of several series of sydnones was undertaken.

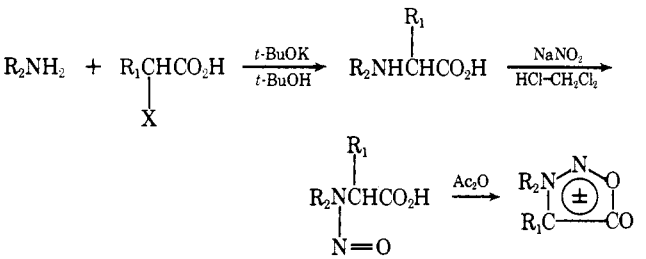


1 2

3

Encouraged by the finding that 3-(*o*-biphenyl)sydnone (1, Ar = 2-biphenylyl; R = H) possesses high antiinflammatory activity, the synthesis of other 3-aryl-4-alkyl (or 4-H) sydnones was carried out (see Scheme I). Pharmacological screening results were disappointing and attention was next focused on the synthesis of 3-aralkyl-4-H or 4-alkylsydnones (2). Again, little activity was noted, and the synthesis of a series of sydnones with a heteroatom in the 3-alkyl side chain (3) was initiated.

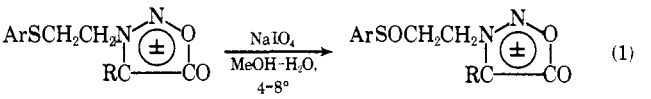
Scheme I



Standard procedures[2] were employed in the preparation of these sydnones. Alkylation of the appropriate amines with α-halo acids yielded the requisite N-substituted amino acids. When the reactions were carried out in *tert*-butyl alcohol with potassium *tert*-butoxide as base, nearly quantitative yields of substituted amino acids were obtained. The amino acids were then treated with sodium nitrite in aqueous hydrochloric acid–methylene chloride and the resulting *N*-nitrosamino acids, without purification, subjected to cyclodehydration in acetic anhydride to yield the desired sydnones.

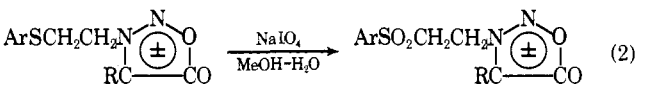
2-Phenylthioethylamine, 2-phenoxyethylamine, and *p*-chlorophenylthio-2-propylamine were prepared by treating the appropriate phenol or thiol with ethanolamine in propionic acid, followed by acidic hydrolysis.[3] Ethylenediamine was treated with tosyl chloride to give 2-aminoethyl-*p*-toluenesulfonamide, and thiophenol reacted with 3-bromopropylamine to give 3-phenylthiopropylamine.[4]

Generation of the sulfoxides 13 and 14 and sulfones 15 and 16 in the presence of the readily oxidized sydnone ring[5,6] was accomplished by treatment with sodium metaperiodate in aqueous methanol. At temperatures of 4–8° the sulfoxide is obtained in quantitative yield (eq 1). At



(1)

ambient temperatures, the sulfone is obtained in comparable yield, even in the presence of excess oxidant (eq 2).

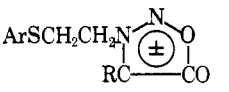


(2)

As can be seen from Table I, a series of potent antiinflammatory sydnones is obtained when the *o*-biphenyl group in 1 is replaced by 2-arylthio and 2-arylsulfoxyethyl substituents. The ED_{50} of 5 mg/kg shown by 10 is equal to hydrocortisone or phenylbutazone in this test. Comparing the sulfides 11 and 12 with the sulfoxides 13 and 14 and sulfones 15 and 16, potency is diminished as the oxidation state increases.

Activity is greatly reduced when sulfur is replaced by oxygen (17) and is eliminated entirely when it is replaced by a sulfonamide (18) or methylene (19) group. Lengthening the ethylene bridge (20) and branching† (21) also adversely affect potency.

Consideration of these results indicates that 22 constitutes a general structure type which can be expected to exhibit potent antiinflammatory activity.



22

Ar = phenyl or substituted phenyl
R = H or CH_3

Studies are in progress to define the structural requirements for activity in greater detail by examining the effects of changes in R and Ar.

Experimental Section‡

General Synthesis of Sydnones. (a) **Alkylation.** The appropriately substituted α-bromoacetic acid (0.1 mol), 2-arylthioethylamine (0.1 mol), and *t*-BuOK (0.1 mol) in 600 ml of *t*-BuOH were heated at reflux under nitrogen overnight. The solvent was removed under reduced pressure and the residue was taken up in 250 ml of 2% aqueous NaOH. The aqueous solution was extracted with Et_2O and acidified to pH 5 with concentrated HCl. Filtration and washing with H_2O gave the amino acid sufficiently pure for nitrosation.

(b) **Nitrosation.** The amino acid (0.08 mol) and $NaNO_2$ (0.09 mol) in 400 ml of 1:1 CH_2Cl_2–H_2O were stirred at 0° while 8 ml of concentrated HCl was added dropwise over a 1-hr period. Stirring

† The presence of a *p*-chloro substituent on the phenyl ring increases antiarthritic activity: H. Wagner and J. Hill, unpublished results.

‡ Melting points were determined in a Thomas-Hoover apparatus and are uncorrected. Elemental analyses, indicated by symbols of the elements, were within ±0.4% of the theoretical values. Ir, uv, and nmr spectra of all new compounds were consistent with proposed structures.