the main variable was the nature of the cotinine used: expt 1, cotinine-d_0; expt 2, cotinine-3,3-d_2 (>99%); expt 3, mixture A (Table I); and expt 4, mixture B (Table I). The description which follows details the procedure followed for mixture B only although it applies to all studies.

A mixture containing cotinine-d_0 (**1a**, 31.2 mg) and cotinine-3,3-d_2 (**1b**, 228 mg) in 13 ml of saline was prepared and 10 ml (200 mg) were administered to a 7-kg male Rhesus monkey by iv infusion over a 10-min period. The pH of the total 24-hr urine collection was adjusted to 9 with 1 *N* NaOH and the resulting solution was extracted continuously with CH_2Cl_2 for 24 hr. Silica gel tlc (EtOH–Me_2CO–C_6H_6–concentrated NH_4OH, 5:40:50:5) of the CH_2Cl_2 residue (100 mg) indicated five major fluorescent spots with R_f values 0.17, 0.27, 0.35, 0.42, and 0.58. These correspond to cotinine *N*-oxide, *trans*-3-hydroxycotinine, desmethylcotinine, 5-hydroxycotinine, and cotinine, respectively.[11] Each band was scraped from the preparative plate and was eluted with EtOH. Mass spectra were obtained by direct insertion with an AEI MS-12 operated at a resolving power of 1000, accelerating potential 8 kV, ionizing potential 50 eV, trap current 500 μA, and source temperature 200°. Low-resolution data reduction was accomplished by a PDP 8/I computer.

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Notes

Triarylhaloethylenes as Gonadotrophin Inhibitors

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In a previous paper[1] we described the synthesis of a series of basic-ether-substituted triarylhaloethylenes as gonadotrophin inhibitors. We now wish to report the synthesis and gonadotrophin-inhibiting activity of a group of compounds related to the former but with more diversifications in structure.

Chemistry. The compounds described herein (Table I) were prepared by two general methods: (A) halogenation of the corresponding triarylethylene according to previously published[1] methods, or (B) etherification of the corresponding phenolic triarylethylene with the appropriately substituted alkyl halide in the presence of base. The yields by either method were comparable and the methods themselves were chosen on a basis of synthetic convenience relative to the availability or ease of attainment of starting materials. The synthesis of the phenanthene 8 required the preparation of 9-*p*-hydroxyphenylphenanthrene.[2] This compound was then etherified with β-diethylaminoethyl chloride (method B) and the product was chlorinated (method A) to give 8.

All of these compounds except 7 were converted to a citrate or hydrochloride salt prior to screening. Efforts were not made to separate geometric isomers where the possibility existed; thus, in such cases, chemical characterization and biological testing were done on a mixture of isomers.

Biology. Table II indicates the gonadotrophin-inhibiting activities of these compounds as determined by the method of Holtkamp, *et al.*[3] A lower mean relative (expressed per 100 g of body weight) ventral prostate weight of the treated rats as compared with that of the controls was used as the index of pituitary gonadotrophin inhibition.

In general, these compounds were gonadotrophin inhibitory at one or more doses in the range 3–50 mg/kg/day. Compounds 1, 4, 6, and 7, however, were more active than the rest. The introduction of an additional substituent such as *p*-*tert*-butyl (2) or β-diethylaminoethoxy (5) diminished the response as did the increase in the size of the amino function to pyrrolidino (3). That increased coplanarity of the rings is detrimental can be seen in the lack of activity in the phenanthrene 8.

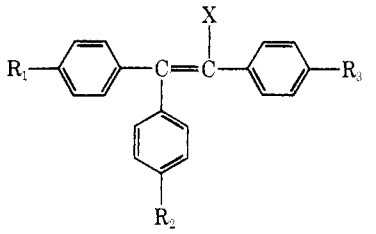
Experimental Section

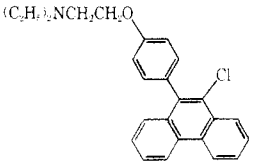
The ethylene starting material for 2 was prepared according to ref 4. Other starting materials are noted below. All melting points were taken on a Thomas-Hoover apparatus. Broad melting points, where they occurred, were attributed to mixtures of cis–trans isomers. All final products analyzed within 0.4% for C, H, and a third element (Cl or N).

***p*-(2-Chloro-1,2-diphenylvinyl)phenol.** A solution of 424 g (1.48 mol) of *p*-(1,2-diphenylvinyl)anisole[5] in CCl_4 was treated with 105 g (1.48 mol) of Cl_2 in CCl_4 over a 2-hr period. After the addition was complete, the solution was heated under reflux for 1 hr and evaporated. The oily residue was treated with petroleum ether (bp 70–90°) and the resulting solid was filtered. Recrystallization from ether gave 178 g of *p*-(2-chloro-1,2-diphenylvinyl)anisole which melted at 104–106°. Condensation of the mother liquor followed by dilution with petroleum ether gave a second crop, 156 g, mp 80–88°, and a third crop, 75 g, mp 78–86°. The total yield was 409 g (86%).

A 119-g (0.37 mol) quantity of the anisole (mp 104–106°) was added at once to 115 g (1.0 mol) of boiling (*ca.* 215°) pyridine hydrochloride. The solution was boiled for 20 min after which it was poured onto ice. The product was extracted with Et_2O to give 75

Table I. Physical Properties of Triarylethylenes



No.	R_1	R_2	R_3	X	Formula[a]	Mp, °C	Method	Yield, %[b]
1	$(CH_3)_2NCH_2CH_2O$	H	H	Cl	$C_{24}H_{24}ClNO$	106–113[c]	B	37
2	$(C_2H_5)_2NCH_2CH_2O$	H	*t*-Bu	Cl	$C_{30}H_{36}ClNO$	117–119[c]	A	54
3	c-$C_4H_8NCH_2CH_2O$	H	H	Cl	$C_{26}H_{26}ClNO$	121–125[c]	B	51
4	H	H	$(C_2H_5)_2NCH_2CH_2O$	Cl	$C_{26}H_{26}ClNO$	120–122[d]	A	25[e]
5	$(C_2H_5)_2NCH_2CH_2O$	$(C_2H_5)_2NCH_2CH_2O$	H	Cl	$C_{32}H_{41}ClN_2O_2$	111–114[f]	A	18
6	$(C_2H_5)_2NCH_2CH_2O$	H	H	CN	$C_{27}H_{28}N_2O$	106–108[c]	B	65
7	$C_2H_5OCH_2CH_2O$	H	H	Cl	$C_{24}H_{23}ClO_2$	51.5–75.0[g]	B	30
8					$C_{26}H_{26}ClNO$	202–204[d]	A	57

[a] All compounds analyzed within 0.4% of theoretical for C, H, and a third element. In some cases a fourth element was analyzed. Analyses were performed on the salt form indicated in the melting point column. [b] Yields are of the final product in the salt form indicated in the melting point column. [c] Dihydrogen citrate salt. [d] Hydrochloride salt. [e] An intermediate dichloro compound was isolated by method A. This intermediate eliminated HCl when heated to 140–150° for 20 min after which the desired product was obtained. [f] Bis(dihydrogen citrate) salt. [g] Initially isolated as an oil which solidified on continued standing.

Table II. Mean Relative Ventral Prostate Weight Changes

Compd no.	Ventral prostate, % of control[a] 3 mg/kg/day	10 mg/kg/day	30 mg/kg/day	50 mg/kg/day
1		44, 31	30	37
2			81	
3		76, 70	59, 82	71
4	35			
5		79, 98	58, 84	41
6		32	26	50
7		32		30
8				110, 112

[a] The ventral prostate weight changes are expressed as per cent of control. Each value is the result of a separate test.

g of material melting at 118–125°. Recrystallization from Et_2O–petroleum ether gave a total of 65.6 g (58%) of material in three crops, mp 122–127°, 117–127°, 112–124°.

3-(*p*-Hydroxyphenyl)-2,3-diphenylacrylonitrile. A 39.0-g (0.125 mol) quantity of *p*-(2-cyano-*cis*-1,2-diphenylvinyl)anisole[6] was added rapidly to 150 g of boiling (*ca.* 215°) pyridine hydrochloride. The hot solution was then poured onto ice and this mixture was extracted with ether. The ethereal solution was extracted with 10% aqueous NaOH which was subsequently acidified. The residue was recrystallized from ether to give 31 g (83%) of product melting at 201–210°. When repeated with the corresponding trans isomer, this procedure gave a product of the same melting range; thus, it appeared that the same cis–trans mixture of phenols resulted in each case.

1,1-Bis[*p*-(β-diethylaminoethoxy)phenyl]-2-phenylethylene. A 28.4 g (0.056 mol) quantity of 1,1-bis[*p*-(β-diethylaminoethoxy)-phenyl]-2-phenylethanol[7] was heated under reflux for 2 hr in 50 ml of concentrated aqueous HCl and 150 ml of EtOH. The solution was condensed *in vacuo*, neutralized with aqueous NaOH, and extracted with Et_2O. The base was taken up in 250 ml of 2-butanone and treated, while hot, with 20.6 g (0.11 mol) of citric acid in 100 ml of MeOH. Upon cooling, 43.3 g (88%) of product separated as the bis(dihydrogen citrate) salt, mp 110–112°.

10-Chloro-9-[*p*-(β-diethylaminoethoxy)phenyl]phenanthrene Hydrochloride. A 66.0-g (0.39 mol) quantity of 2-methylbiphenyl[8] was heated under reflux with 78.0 g (0.44 mol) of *N*-bromosuccinimide in 1 l. of CCl_4 for 4 hr. The mixture was then filtered and evaporated. The residue was taken up in Et_2O and this solution was dried over $MgSO_4$, filtered, and distilled to give 58.0 g (60%) of 2-bromoethylbiphenyl: bp 133° (1.2 mm); $n^{25}D$ 1.6221.

A Grignard of 17.3 g (0.07 mol) of the latter was prepared in 100 ml of Et_2O. This solution was then added to an Et_2O solution of 6.0 g (0.05 mol) of anisonitrile after which the reaction mixture was poured onto ice–concentrated HCl. The layers were separated and the aqueous phase was heated on the steam bath overnight. The product (*p*-methoxy-α-*o*-biphenylacetophenone) was obtained as an oil (8.3 g, 55%) by Et_2O extraction followed by chromatography on alumina.

After the entire sample of this ketone was heated under reflux for 24 hr in 100 ml of HOAc and 60 ml of 48% aqueous HBr, the reaction mixture was poured onto ice. The oil which separated was extracted with Et_2O and the organic layer was then extracted with 10% aqueous NaOH. The aqueous layers were then acidified and extracted with Et_2O to give the crude product. It was recrystallized from C_6H_6–petroleum ether (bp 75–90°) to give 3.3 g (45%) of 9-*p*-hydroxyphenylphenanthrene, mp 151–153°. No depression of melting point occurred when this product was mixed with an authentic sample prepared as described by Bradsher.[2]

The entire sample of 9-*p*-hydroxyphenylphenanthrene was heated under reflux for 2 hr with 1.7 g (0.0125 mol) of β-diethylaminoethyl chloride and 10 g of K_2CO_3 in 150 ml of 2-butanone. The mixture was cooled, treated with Et_2O, and filtered. The solvents were evaporated and the oily residue was converted to the hydrochloride salt by treatment with HCl in Et_2O–EtOH. After recrystallization from MeOH the 9-[*p*-(β-diethylaminoethoxy)phenyl]-phenanthrene hydrochloride weighed 2.7 g (54%) and melted at 207–208°.

This material was chlorinated according to method A to give

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.

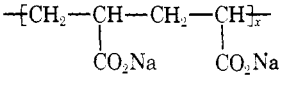
Toxicity and Structure. Since these polymers were administered at the maximum tolerated level, the largest doses used (as shown in Tables I and II) are some indication of their toxicities. For a given series of compounds

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

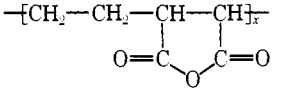
[a] These compounds have a range of molecular weights that is usual for most unfractionated polymers. [b] Four ip injections were given to six rats at 24-hr intervals, starting on the third day after the solid tumor was introduced beneath the skin of the rat. [c] Animals were sacrificed on day 7 and 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