TABLE I

THE ANTIANDROGENIC ACTIVITY OF 17α-ETHYL-17β-METHYL-$\Delta^{4,13}$-GONADIEN-3-ONE (III)

Expt.	Test compd. designation	Total dose, mg. s.c.	Total dose of testosterone, mg.	No. of mice	Mean body wt., g.	Tissue ratio ± S. E. Prostate	Tissue ratio ± S. E. Seminal vesicles
A	0	0	0	8	17	0.02 ± 0.005	0.17 ± 0.016
		0	0.8	10	17	0.19 ± 0.014	1.24 ± 0.153
	Progesterone	5	0.8	9	17	0.14 ± 0.016	0.83 ± 0.059
		10	0.8	9	17	0.15 ± 0.012	0.68 ± 0.125
		20	0.8	8	15	0.12 ± 0.013	0.42 ± 0.032
	III	17	0.8	10	16	0.13 ± 0.009	0.53 ± 0.026
B	0	0	0	9	18	0.06 ± 0.015	0.23 ± 0.018
		0	0.8	10	16	0.23 ± 0.020	0.94 ± 0.133
	Progesterone	5	0.8	9	16	0.16 ± 0.022	0.84 ± 0.100
		10	0.8	9	16	0.11 ± 0.021	0.55 ± 0.076
		20	0.8	10	16	0.12 ± 0.010	0.43 ± 0.040
	III	5	0.8	7	18	0.15 ± 0.015	0.59 ± 0.045
		10	0.8	8	15	0.15 ± 0.021	0.62 ± 0.048

Biological Activity. Methods.—Antiestrogenic activity was assessed by the method of Dorfman, *et al.*[3] This test measures the ability of a compound to inhibit the uterotrophic activity of estrone. Antiandrogenic activity was determined in the testosterone-stimulated castrated mouse.[4] The end points were the weights of the seminal vesicles and prostate. The compound was also tested for possible antitumor activity using a transplantable rat fibroadenoma system.[5]

Results and Conclusion

The antiandrogenic activity of III is illustrated in Table I. The compound was assayed twice in parallel with the standard progesterone at 5, 10, and 20 mg. total doses. Statistically significant decreases in the seminal vesicles' response were observed after giving III at total doses of 5, 10, and 17 mg. The dimethyl derivative (V) was active at the 20 and 40 mg. dose levels (Table II).

As indicated in Table III, antiestrogenic activity was demonstrated at all three total doses studied, 50, 125, and 500 γ. The maximum inhibition was 56% at the highest doses. This compares (Table II) with the antiestrogenic activity of V which showed a maximum activity of 49%, and 160 γ was the minimum effective dose. It is apparent from Table I and III that no meaningful dose–response curves can be drawn for III in either the antiandrogenic or antiestrogenic assay. At 14 mg. total dose, the compound did not inhibit the mammary tumor weight nor the glycine-2-C^{14} incorporation into the tumor proteins.

The 17-ethyl derivative (III) is perhaps more active as an antiandrogen than the 17-methyl compound (V) under the conditions studied. Antiestrogenic activity of the two compounds is of the same order.

TABLE II

COMPARATIVE BIOLOGICAL ACTIVITY OF THE 17,17-DIMETHYL (V) AND 17α-ETHYL-17β-METHYL (III) DERIVATIVES OF $\Delta^{4,13}$-GONADIEN-3-ONE

Compd.	Test	Result, s.c. injection	Ref.
III	Antiandrogen	+ at 5, 10, 17, and 20 mg.	This report
V	Antiandrogen	+ at 20 and 40 mg.	1
III	Antiestrogen	+ at 50, 125, and 500 γ Maximum inhibition, 56%	This report
V	Antiestrogen	+ at 160–1500 γ Maximum inhibition, 49%	1
III	Antimammary tumor assay	− at 14 mg.	5

TABLE III

THE ANTIESTROGENIC ACTIVITY OF 17α-ETHYL-17β-METHYL-$\Delta^{4,13}$-GONADIEN-3-ONE (III)

Test compd. designation	Total dose, γ s.c.	Total dose of estrone, γ	No. of mice	Mean uterine ratio ± S. E.
0	0		10	0.97 ± 0.053
	0	0.4	10	5.09 ± 0.354
III	50	0.4	10	3.64 ± 0.281
	125	0.4	10	4.74 ± 0.322
	500	0.4	10	2.76 ± 0.240

(3) R. I. Dorfman, F. A. Kincl, and H. J. Ringold, *Endocrinology*, **68**, 17 (1961).
(4) R. I. Dorfman, *Proc. Soc. Exptl. Biol. Med.*, **111**, 441 (1962).
(5) O. Abe, A. Herranen, and R. I. Dorfman, *ibid.*, **111**, 706 (1962).

Hydroxy-, Nitro-, Amino-, and Methoxy-4-(4-dimethylaminostyryl)quinolines[1]

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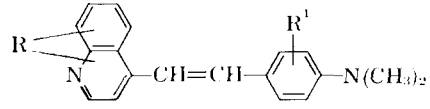
In view of the fact that *in vivo* hydroxylation of aromatic rings is one of the possible metabolic changes in drugs,[2] we have prepared a series of hydroxy derivatives of 4-(4-dimethylaminostyryl)quinolines (Table I) in order to learn whether the toxicity of these compounds and their antitumor action are greater or less than

(1) This work was supported in part by grants from the American Cancer Society and Public Health Service Research Grants CA 03717-01 through 03717-07 from the National Cancer Institute.
(2) R. T. Williams, "Detoxication Mechanisms," John Wiley and Sons, Inc., New York, N. Y., 1959, pp. 190, 430, 456.

TABLE I

HYDROXY-, NITRO-, AMINO-, METHOXY-, AND ALKYLDIMETHYLAMINOSTYRYLQUINOLINES



No.	Substituent, R or R′	M.p.,[a] °C.	Reaction[b] Temp., °C.	Time, hr.	Yield, %	Formula	Calcd., % C	H	Found, % C	H	T/C[c] @ mg./kg.	Deaths @ mg./kg.	ED₅₀,[d] γ/ml.
1	2′-OH[e]	219–220	150–155	1	39	$C_{19}H_{18}N_2O$	78.58	6.25	78.41	6.49[f]	1 150	1/3 150	
2	3′-OH	139–140	*g*		3	$C_{19}H_{18}N_2O$	78.58	6.25	78.55	6.54[f]			2
3	5(or 7-)-OH	302–304	200–210	0.33	11	$C_{19}H_{18}N_2O$	78.58	6.25	78.68	6.29[h]	1 50	0 100	
4	6-OH[i]	292–294	180–190	0.33	33	$C_{19}H_{18}N_2O$	78.58	6.25	78.29	6.32[f]	1 250		
5	8-OH	228–230	190–200	0.33	43	$C_{19}H_{18}N_2O$	78.58	6.25	78.63	6.23[f]	0.6 100		
6	5,8-$(OH)_2$	238–240	135–137	4	27	$C_{19}H_{18}N_2O_2$	74.46	5.92	74.43	6.15[f]	0.7 50		
7	3′-NO_2	128–130	140–150	10	18	$C_{19}H_{17}N_3O_2$	71.45	5.37	70.75	5.28[f,j]			
8	6-NO_2	214–216	100–110	0.167		$C_{19}H_{17}N_3O_2$	71.45	5.37	71.40	5.47[h]			
9	8-NO_2	208–209	105–110	0.133	70	$C_{19}H_{17}N_3O_2$	71.45	5.37	71.31	5.32[f]	1 100	0/3 200	
10	3′-NH_2	129–130	*l*		62	$C_{19}H_{19}N_3$	78.85	6.63	78.50	6.75[f]			2
11	2-$N(CH_3)_2$	158–160	180	1		$C_{21}H_{23}N_3$	79.47	7.28	79.28	7.38[h,k]	0.21 50	1/3 100	0.3
12	8-NH_2	209–211	*l*		46	$C_{19}H_{19}N_3$	78.85	6.63	78.67	6.52[f]	0.2 100		
13	2′-CH_3O	137–140	150–160	1	41	$C_{20}H_{20}N_2O$	78.90	6.62	78.65	6.40[f]	0.013 25	1/3 50	5
14	3′-CH_3O[m]	120	145–158	0.5	4	$C_{20}H_{20}N_2O$	78.90	6.62	78.55	6.54[f]			3
15	3′-CH_3O[n]	170–171	140–160	2	1	$C_{20}H_{20}N_2O$	78.90	6.62	79.02	6.54[f]			0.7
16	2′,5′-$(CH_3O)_2$	114–116	140–160	1	1	$C_{21}H_{22}N_2O$	75.42	6.63	75.50	6.71[f]	1 100		
17	5(or 7)-CH_3O	152–154	115–125	0.5		$C_{20}H_{20}N_2O$	78.90	6.62	79.02	6.58[h]	0.13 50	3/3 100	
18	6-CH_3O[o]	142	160–170	1.5							0.20 50	6/6 100	
19	6-CH_3O	206–208	120–130	0.75		$C_{20}H_{20}N_2O$	78.90	6.62	78.95	6.47[f]	0.20 50	6/6 100	
20	5,8-$(CH_3O)_2$	177.5–178.5	150–170	0.5		$C_{21}H_{22}N_2O$	75.45	6.59	75.28	6.59[f]	0.25 40	3/3 80	
21	2′,3′-$(CH_2)_3$ (*cis*)[p]	155.0–156.0	140–158	2	0.2	$C_{21}H_{22}N_2$	83.40	7.33	83.13	7.22[h]			5
22	2′,3′-$(CH_2)_3$ (*trans*)[q]	216–217	140–158	2	0.2	$C_{21}H_{22}N_2$	83.40	7.33	83.24	7.20[h]			5
23	3′-C_2H_5	124.0–125.0	140–150	1	21	$C_{21}H_{22}N_2$	83.40	7.33	83.15	7.34[f]	0.05 12.5	1/3 25	1
24	3′-NO_2[r]	194–195	180–190	5.5	16	$C_{18}H_{15}N_3O_2$	70.80	4.95	70.17	5.20[f,s,e]			
25	3′-NH_2[r]	194–195	*l*		15	$C_{18}H_{17}N_3$	78.51	6.22	78.71	6.43[f]			6
26	3′-OH[t]	51–53	*g*			$C_{18}H_{16}N_2O$	78.23	5.84	78.81	6.38[h]			3

[a] Determined by use of a Thiele tube; corrected for thermometer stem exposure. [b] Heating equimolar amounts of aldehyde and lepidine hydrochloride. [c] We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor-bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts were reported as the ratio *T/C*. [d] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at University of Miami Cell Culture Laboratory and Southern Research Institute. [e] 2′, 3′, and 5′ indicate positions on the benzene ring of the styryl group. [f] Analysis by Galbraith Microanalytical Laboratories. [g] Prepared by diazotization of 4-(4-dimethylamino-3-aminostyryl)quinoline, then hydrolysis. [h] Analysis by Weiler and Strauss, Oxford, England. [i] M. A. Clapp and R. S. Tipson, *J. Am. Chem. Soc.*, **68**, 1332 (1946), gave m.p. 280–282° for this 6-hydroxy compound and m.p. 110° for this 6-methoxy compound. [j] Note that these analyses were not satisfactory even after repeated recrystallization of the nitro compound. [k] *Anal.* Calcd.: N, 13.25. Found: 13.03.[c] [l] Prepared by stannous chloride reduction of the nitro compound. [m] Probably the *cis* form, λ_{max}^{MeOH} about 370 mμ. [n] Probably the *trans* form, λ_{max}^{MeOH} about 410 mμ. [o] These compounds contained a monomethylamino group instead of a dimethylamino group. [p] *Anal.* Calcd.: N, 13.76. Found: N, 12.90.

those of the parent substance.[3] One of the routes chosen was the preparation of nitro compounds, reduction to the amino compounds, diazotization, and finally replacement of the diazonium group by hydroxyl. One of the methyls in the dimethylamino group was split off in some cases to form monomethylamino compounds. The compositions of the latter compounds differed so little from the expected dimethylamino compound that the loss of the methyl group was detected by infrared or n.m.r. observations, not by analytical data.[4] In a few instances it was found convenient to couple a hydroxylepidine with a suitable aldehyde or to prepare a methoxy dimethylaminostyrylquinoline and hydrolyze it to the hydroxy compound. Some 4-(4-dimethylamino-2-methoxystyryl)-quinoline was recovered unhydrolyzed after 4 hr. in boiling sulfuric acid (60% by wt.) along with a small yield of the desired 2-hydroxy product. The latter was obtained more conveniently from 4-dimethylamino-2-hydroxybenzaldehyde and lepidine hydrochloride.

Like many other hydroxy heterocyclic substances[5] most of these hydroxy compounds were not readily soluble in oil. Perhaps for this reason, they did not show much biological activity when administered in this way. It is interesting to note that the melting points of the 3′-amino and 3′-hydroxy compounds were lower than those of the other hydroxy and amino compounds listed. Fifteen of the compounds listed in Table I were tested by single i.p. injection in oil, against Walker 256 tumors in rats, at the Chester Beatty Research Institute. None of the hydroxy compounds tested showed antitumor activity, but the 8-amino, the 2-dimethylamino, and all four monomethoxy compounds showed clear-cut activity. The most potent of the fifteen compounds were the 3′-ethyl and the 2′-methoxy.

Experimental

All styryl compounds were prepared by reaction of the aldehyde with the lepidine hydrochloride unless otherwise indicated.

2-Dimethylaminolepidine Hydrochloride.—A solution of 2-chlorolepidine hydrochloride in dimethylformamide was refluxed for 3 hr. The white crystals that separated on cooling were collected and dried; m.p. 330°. This material was used for the preparation of **11**.

Anal. Calcd. for $C_{12}H_{14}N_2 \cdot HCl$: neut. equiv., 222.7. Found: neut. equiv. (titration using pH meter), 223.0.

4-(4-Hydroxystyryl)quinoline.—A solution of 2.5 g. (0.01 mole) of 4-(4-aminostyryl)quinoline in 500 ml. of 40% H_2SO_4 was diazotized with 1.09 g. of sodium nitrite (0.01 mole) at 0–5° The diazotized salt was slowly added to boiling water, then the solution was boiled an additional 5 min. The acid mixture was neutralized using NaOH initially and completing the neutralization with Na_2CO_3. The precipitate was filtered, dried, and recrystallized from octane until the compound appeared chromatographically pure; m.p. 259–261°, yield 0.58 g. (23%).

Anal. Calcd. for $C_{17}H_{13}NO$: C, 82.55; H, 5.71. Found: C, 82.42, 82.37; H, 5.5, 5.7.

(3) C. T. Bahner, L. M. Rives, E. B. Senter, W. Longmire, H. Kinder, D. B. Bales, F. Hannan, B. Pettyjohn, W. K. Easley, L. Free, and H. Free, *J. Org. Chem.*, **27**, 2233 (1962); C. T. Bahner, *Acta Unio Intern. Contra Cancrum*, **20**, 253 (1964).

(4) We are grateful to Dr. Clarence Cook of the Research Triangle Institute for obtaining and interpreting infrared and n.m.r. curves on selected compounds.

(5) A. Albert, "Heterocyclic Chemistry," University of London, London, 1959, p. 44.

Synthesis and Pharmacological Study of New Piperazine Derivatives. III. Phenoxyalkylpiperazines

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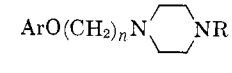
Early pharmacologic studies demonstrated that phenoxyethylamine and some of its derivatives were sympatholytic.[1] In our search for new structures with such useful activities,[2] it was considered of interest to introduce phenoxyethyl and more generally aryloxyalkyl groupings into the 4-position of a series of 1-phenylpiperazines and some of their congeners. In this investigation, we decided to prepare unsymmetrical piperazines (I) and to vary the substituents Ar and R, and the size of the alkylene unit. The following compounds were prepared.



I, Ar = phenyl, *p*-chlorophenyl, *p*-fluorophenyl, *o*-tolyl, *p*-methoxyphenyl, 2,6-xylyl, 3,4-dimethoxyphenyl
R = phenyl, *o*- or *p*-chlorophenyl, *o*-methoxyphenyl, 2,3-xylyl, 2-pyridyl
n = 2 to 10

To our knowledge, the synthesis of only one compound of formula I (Ar = R = C_6H_5; n = 2) has been reported[3]; recently Abood and co-workers[4] have synthesized and tested for their psychotropic properties, a large number of aryloxyalkylpiperazines of type I but with R = H or $CO_2C_2H_5$ (n = 2 to 7).

In Table I descriptive and analytical data are listed for compounds of formula I. They were obtained from the corresponding aryloxyalkyl bromides by treatment with the appropriate monosubstituted piperazine in the presence of anhydrous potassium carbonate in butanol. The desired products were isolated either directly as bases or the bases were converted to hydrochloride salts.

The aryloxyalkyl bromides required (Table II) were prepared from phenoxides and polymethylene dibromides according to previously reported procedures.[5] The use of water as a reaction medium led to the formation of symmetrical bisaryloxyalkanes as side products (method A). No side product was isolated when absolute ethanol and excess dibromide (method B) was used. Synthetic details are given for these methods

(1) D. Bovet and F. Bovet-Nitti, "Medicaments du système nerveux végétatif," S. Karger, S.A., Bâle, 1948, p. 222.

(2) Previous paper in this series: R. Ratouis, J. R. Boissier, and C. Dumont, *J. Med. Chem.*, **8**, 104 (1965).

(3) A. P. Swain and S. K. Naegele, *J. Am. Chem. Soc.*, **76**, 5091 (1954).

(4) L. G. Abood, L. Brady, E. Boulton, V. Lipman, and M. Fishman, *Arch. Intern. Pharmacodyn.*, **134**, 106 (1961).

(5) See for examples Table II, footnotes *c* to *i*.