sion was kept below 10° and treated with $NaNO_2$ (25.4 g., 0.368 mole, in 100 ml. of water). The mixture was allowed to warm to room temperature and stirred for a further 2 hr. It was filtered, and the organic layer of the filtrate was dried and evaporated to leave the crude nitrosamine. The solid filtered off was the hydrochloride of the starting material, which was recycled as above. The nitrosamine (total yield 85.4 g., 81%) had b.p. 172–174° (0.4 mm.), $n^{20}D$ 1.5618. The structure was confirmed by the strong absorption at 1480–1460 cm.$^{-1}$ and lack of N–H absorption in the infrared spectrum.

N-Benzyl-N-2-(2,6-dimethylphenoxy)ethylhydrazine (VII).—The above nitrosamine (37 g., 0.13 mole) was reduced in the normal manner with $LiAlH_4$ (6.84 g., 0.18 mole) in ether. After decomposition of the reaction complex, the product was isolated by extraction from the organic phase with dilute HCl, and subsequent basification. Distillation yielded 18.3 g. (52%) of the hydrazine, b.p. 146–149° (0.2 mm.), $n^{19}D$ 1.5584.

Anal. Calcd. for $C_{17}H_{22}N_2O$: N, 10.36. Found: N, 10.13.

N-Benzyl-N-guanidino-2-(2,6-dimethylphenoxy)ethylamine Sulfate.—The above hydrazine (10.8 g., 0.04 mole) and 1-amidino-3,5-dimethylpyrazole sulfate[12] (7.48 g., 0.04 mole) were heated under reflux in aqueous ethanol for 5 hr. The solvent was evaporated and the residue, after being washed with ether, was crystallized from methanol–ether to give 6.8 g. (47%) of product, m.p. 100–102°.

Anal. Calcd. for $C_{36}H_{50}N_8O_6S$: C, 59.84; H, 6.95; N, 15.52. Found: C, 59.66; H, 7.00; N, 14.98.

2-(2,6-Dimethylphenoxy)ethylaminoguanidine Sulfate (IVb).—The above N-benzylaminoguanidine (3.0 g.) was hydrogenated with palladium–charcoal catalyst in acetic acid (100 ml.) at room temperature and pressure. After filtration of the catalyst and evaporation of the solvent, the residue was recrystallized from water to give the product (0.9 g., 40%), m.p. 213°, undepressed on admixture with the product from the reaction of 2-(2,6-dimethylphenoxy)ethylhydrazine and S-methylisothiourea sulfate. The infrared spectra and chromatographic characteristics of the two products were identical.

N-[2-(2,6-Dichlorophenoxy)ethyl]-N-cyanohydrazine (IX).—2-(2,6-Dichlorophenoxy)ethylhydrazine (20.5 g., 0.093 mole) was added dropwise over 30 min. to a stirred solution of cyanogen bromide (9.8 g., 0.093 mole) in ethanol (60 ml.) and water (200 ml.). The mixture was stirred at room temperature for 1.5 hr. and then extracted with ether. The ethereal extract was washed with aqueous 5 *N* NaOH and with water and, after drying, was evaporated to leave an oil (14.5 g., 63%), ν_{max} 2220 cm.$^{-1}$; ***o*-nitrobenzal derivative,** m.p. 116.5–117° (from methanol).

Anal. Calcd. for $C_{16}H_{12}Cl_2N_4O_3$: C, 50.69; H, 3.19; Cl, 18.70; N, 10.78. Found: C, 50.59; H, 3.06; Cl, 18.91; N, 10.47.

1-Amino-1-[2-(2,6-dichlorophenoxy)ethyl]guanidine (V).—The cyanohydrazine IX (4.92 g., 0.02 mole) and ammonium sulfate (2.64 g., 0.02 mole) were heated under reflux in aqueous ammonia (10 *N*, 50 ml.) and 2-propanol (25 ml.) for 3 hr. The mixture was allowed to cool, and the upper layer was decanted from the oil which had been deposited. The decanted liquor was evaporated to dryness, and the product was obtained from this residue by extraction with hot methanol. Recrystallization from methanol gave the pure product: m.p. 215°; m.m.p. 195–205° with IVa; ν_{max} 1680, 1670, and 1645 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{26}Cl_4N_8O_6S$: C, 34.62; H, 4.20; N, 17.95. Found: C, 34.44; H, 4.35; N, 17.98.

Benzal derivative had m.p. 189–190° (from ethanol–water).

Anal. Calcd. for $C_{32}H_{34}Cl_4N_8O_6S$: C, 48.00; H, 4.28; Cl, 17.71; N, 14.01. Found: C, 47.71; H, 4.39; Cl, 17.51; N, 14.28.

Degradative Experiments.—2-(2,6-Dimethylphenoxy)ethylaminoguanidine (IVb, 3.0 g.) and Raney nickel (15 g.) in methanol (100 ml.) were heated under reflux for 6 hr. The nickel was filtered off, and the filtrate was evaporated to dryness. The residue was extracted with ether to leave a solid (0.8 g.), m.p. 285–290° (undepressed on admixture with guanidine sulfate), the infrared spectrum of which was identical with that of guanidine sulfate. The ethereal extract was evaporated to leave an oil, the infrared spectrum of which was virtually identical with that of 2-(2,6-dimethylphenoxy)ethylamine.[13]

The dichloro analog IVa, when treated in the same manner with Raney nickel, gave 37% guanidine sulfate (mixture melting point and infrared spectrum), and an oil from which no pure product could be isolated. Analytical figures indicated partial loss of chlorine (*Anal.* Calcd. for $C_8H_9Cl_2NO$: Cl, 34.41. Found: Cl, 21.73.).

1-Amino-3-[2-(2,6-dichlorophenoxy)ethyl]guanidine Hydriodide (X).—2-(2,6-Dichlorophenoxy)ethylamine[10] (4.0 g., 0.0195 mole) and S-methylisothiosemicarbazide hydriodide (4.54 g., 0.0195 mole) were heated under reflux in methanol (25 ml.) for 6 hr. The solution was concentrated, and water was added until crystallization occurred. The product (3.5 g., 46%) had m.p. 170°.

Anal. Calcd. for $C_9H_{13}Cl_2IN_4O$: C, 27.65; H, 3.35; N, 14.33. Found: C, 27.94; H, 3.27; N, 14.06.

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(12) J. Augstein, S. M. Green, A. M. Monro, G. W. H. Potter, C. R. Worthing, and T. I. Wrigley, *J. Med. Chem.*, to be published.

(13) D. I. Barron, P. M. G. Bavin, G. J. Durant, I. L. Natoff, R. G. W. Spickett, and D. K. Vallance, *ibid.*, **6**, 705 (1963).

Styrylquinoline Analogs from Heterocyclic Carboxaldehydes[1]

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Indole-3-carboxaldehyde, pyridine-3-carboxaldehyde, thiophene-3-carboxaldehyde, and N-methyl-1,2,3,4-tetrahydroquinoline-6-carboxaldehyde were used instead of *p*-dimethylaminobenzaldehyde to prepare a series of quinoline and isoquinoline derivatives for use in the study of relation of structure to antitumor activity. Data on preparation and properties of the products are shown in Table I.

It has been suspected that biological activity of stilbenes[2] and styrylquinolines[3] depends on resonance involving the ethylene double bond, and therefore could be correlated with an ultraviolet absorption maximum at 380–420 mμ in methanol solution, but it will be noted that several of the ethylene compounds which do not have an absorption peak in this range have ED_{50} as low as 3–6 γ/ml. Results obtained with these compounds encouraged the preparation and testing of compounds containing two or more indole groups without any quinoline ring. Most of these compounds, shown in Table II, had been prepared by others but apparently not tested against tumor cells *in vitro*. It is apparent that the compounds in which the two ring systems are joined through a double bond were much more active in inhibiting tumor cell growth *in vitro* than were those which did not have such a bond. Compound **3** may be an exception to this rule, but Kiang and Mann[4] have suggested that the double bond in this compound may be between the two exocyclic carbon atoms.

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(2) A. Haddow, R. J. C. Harris, G. A. R. Kon, and E. M. F. Roe, *Phil. Trans. Roy. Soc. London*, **A241**, 147 (1948).

(3) C. T. Bahner, *Acta Unio Intern. Contra Cancrum*, **20**, 253 (1964); C. T. Bahner, L. M. Rives, and C. Breder, *J. Med. Chem.*, **7**, 818 (1964); M. Hamana and H. Noda, *Yakugaku Zasshi*, **83**, 342 (1963).

(4) A. K. Kiang and F. G. Mann, *J. Chem. Soc.*, 594 (1953).

TABLE I

ETHYLENE DERIVATIVES

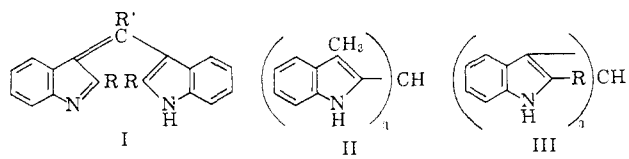
YCH=CHZ

No.	Y	Z	Method	M.p.,[a] °C.	Yield, %	Formula	Ultraviolet absorption λ_{max}, mμ (log ε) In methanol	In acetic acid	Calcd., %: C	H	Found,[b] %: C	H	ED_{50},[c] γ/ml.	T/C (mg./kg.)[d]
1	3-Indolyl	4-Quinolyl	A	241–243[e,f]	30	$C_{19}H_{14}N_2$	380 (4.3) 495	480 (4.5)	84.42	5.22	84.30	5.27	3	
2	3-Indolyl	2-Quinolyl	A	212–213[g,f]	49	$C_{19}H_{14}N_2$	375 (4.4)	465 (4.6)	84.42	5.22	84.38	5.19	4	1 (1600)
3	3-Indolyl	1-Isoquinolyl	A	239–241[e,f]	45	$C_{19}H_{14}N_2$	385 (3.6)	465 (4.0)	84.42	5.22	84.30	5.45	10	1 (1600)
4	N-Methyl-3-indolyl	4-Quinolyl	B	172–173[h,i]	4	$C_{20}H_{17}N_2$	380 (3.9) 495 (3.8)	485 (4.5)	84.20	6.00	83.91	5.63	2	
5	3-Pyridyl	4-Quinolyl	A	109–110[j,k]	2	$C_{16}H_{12}N_2$	325 (4.3)	325 (4.2)	82.72	5.21	82.64	5.66	72	
6	3-Pyridyl	2-Quinolyl	A	96[j,l]	55	$C_{16}H_{12}N_2$	325 (4.4)	350 (4.4)	82.72	5.21	82.54	5.11	3	
7	2-Thiophenyl	4-Quinolyl	B[m]	86[f,n]	24	$C_{15}H_{11}NS$	340 (4.0)	317 (3.9) 495 (3.8)	75.95	4.64	76.08	4.77	6	1 (100)[o]
8	6-(N-Methyl-1,2,3,4-tetrahydroquinolyl)	4-Quinolyl	B[p]	137[q]	25	$C_{21}H_{20}N_2$	415 (4.2)	545 (4.5)	84.00	6.66	84.50	6.80	1	

[a] Corrected for thermometer stem exposure; determined with Thiele tube. [b] Average of two determination by Weiler and Strauss, Oxford, England. [c] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory and Southern Research Institute. [d] 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 sacrified approximately 8 days later and the average weights of tumors in treated and untreated hosts are reported as the ratio *T/C*. [e] Recrystallized from ethanol. [f] Yellow crystals. [g] Recrystallized from methanol. [h] Recrystallized from isooctane and from ethanol. [i] Tan crystals. [j] Recrystallized from isopropyl ether. [k] White crystals. [l] Recrystallized from isohexane. [m] Reacted 0.5 hr. at 130°. [n] Recrystallized from isooctane. [o] Killed 1 of 3 rats at 200 mg./kg. [p] Reaction temperature 155–165°; time, 2 hr.

TABLE II

INDOLE DERIVATIVES



I II III

No.	Compd. type	R	R'	ED_{50}[a]	T/C[b]	@ mg./kg.
1	I	H	H[c]	3	0.7	80[d]
2	I	CH_3	H[e]	2	1.1	80[f]
3	I	CH_3	CH_3[g]	55	1.0	320[h]
4	II	...	...[i]	33	0.7	1600
5	III	H	H	100	1.2	1600
6	III	CH_3	CH_3[j]	30	1.0	1600

[a] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory and Southern Research Institute. [b] See footnote *d*, Table I. [c] W. König, *J. prakt. Chem.*, **84,** 194 (1911). [d] Killed 3/3 at 160 mg./kg. [e] M. Scholtz, *Ber.*, **46,** 2138 (1913). [f] Killed 2/3 at 160 mg./kg. [g] A. K. Kiang and F. G. Mann, *J. Chem. Soc.*, 594 (1953). [h] Killed 2/2 at 625 mg./kg. [i] H. V. Dobeneck and H. Pritzel, *Z. physiol. Chem.*, **299,** 214 (1955). [j] M. Passerini, *Gazz. chim. ital.*, **68,** 480 (1938).

Experimental

The methods of synthesis of compounds in Table I are illustrated below.

Method A.—A mixture of 10.0 g. of indole-3-carboxaldehyde and 12 g. of 2-methylquinoline was heated 21 hr. in a closed container at 125–135°. The hot mixture was then triturated with 150 ml. of isopropyl alcohol. After recrystallization from methanol and from ethanol the yellow crystals melted at 210–211°; yield 8.9 g.

Method B.—A mixture of 7.0 g. of N-methylindole-3-carboxaldehyde and 12.5 g. of lepidine hydrochloride was heated at 160–170° until it solidified (2 hr.). The solid was dissolved in methanol and the solution was neutralized by addition of 8 *N* NaOH, then diluted with water and cooled. The solid was recrystallized once from octane and three times from methanol to yield 2.9 g. of tan crystals, m.p. 172–173°.

Method A tended to give a cleaner product, but only method B was successful with N-methylindole-3-carboxaldehyde, and neither method was satisfactory for N-acetylindole-3-carboxaldehyde.

3,3′,3″-Triindolylmethane.—A solution of 0.10 mole of indole-3-carboxaldehyde, 0.20 mole of indole, and 1 drop of concentrated HCl in 200 ml. of methanol was boiled under reflux for 0.6 hr. The solid product was recrystallized from benzene to give white crystals, m.p. 254–256° cor., yield 80%.

Anal.[5] Calcd. for $C_{25}H_{19}N_3$: C, 83.07; H, 5.30. Found: C, 82.98; H, 5.24.

(5) Analysis by Galbraith Laboratories.

α- and γ-Glutamyl Derivatives of Aminobenzoic Acids

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A previous report assigned the γ-glutamyl structure to the *o*-, *m*-, and *p*-(L-glutamylamino)benzoic acids prepared by condensation of N-carbobenzoxy-L-glutamic anhydride with the methyl esters of the appropriate aminobenzoic acids and subsequent removal of the protecting groups.[1b] The γ-amide I rather than the α-amide II structure was favored because with ninhydrin the materials gave a strong purple color, which was taken to be characteristic of α-amino acids having a free carboxyl group, and because, with phenylisocyanate, derivatives which appeared to be hydrates of hydantoins were obtained. A reinvestigation of the

(1) (a) Predoctoral Fellow (1F1 GM-19,443-01), National Institute of General Medical Sciences. (b) D. L. Ross, C. G. Skinner, and W. Shive, *J. Med. Chem.*, **6,** 208 (1963).