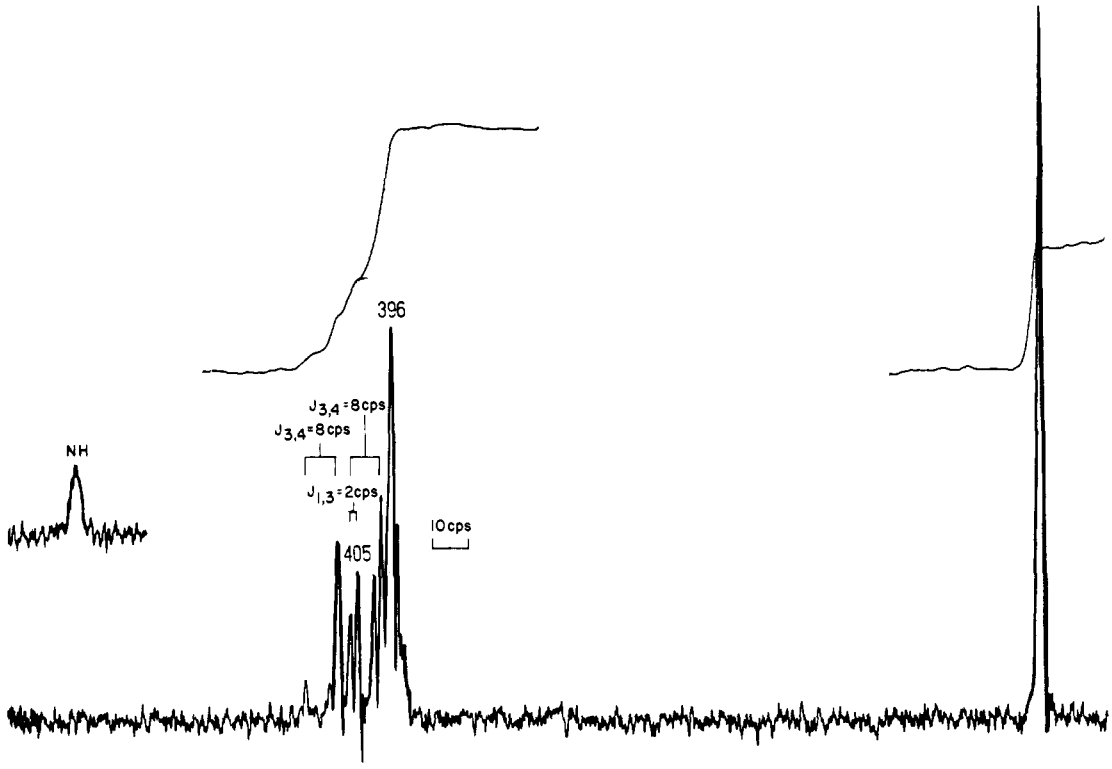

Figure 6.—N.m.r. spectrum (60 Mc.) of 2-chloro-7-methoxyphenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

The Synthesis, Proof of Structure, and Biological Activity of Some Monosubstituted Aminoguanidines[1]

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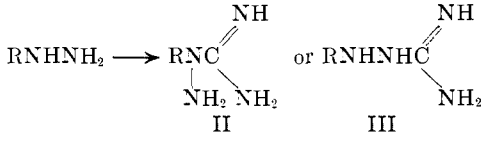
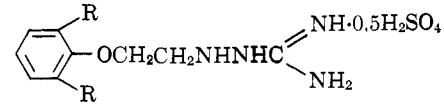
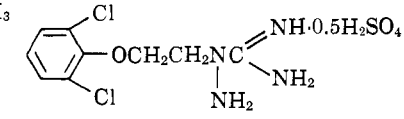
The reaction of monosubstituted hydrazines with S-methylisothiourea sulfate (I) has been claimed[2] to yield substituted aminoguanidines of type II. We have found that 2-(2,6-disubstituted phenoxy)ethylhydrazines react with I to give, as the main product, aminoguanidines of type III.



Reaction of 2-(2,6-dichlorophenoxy)ethylhydrazine with I yielded a compound which was assigned structure IVa,[3] on the basis of its failure to give a benzal derivative. As this compound (guanoclor[4]) displayed both dopamine β-oxidase inhibitory and antihypertensive properties,[5] it was of interest to synthesize the isomer



IVa, R = Cl
b, R = CH_3



V

V and examine its biological properties. Comparison of this compound with IVa and the analog IVb, the latter two synthesized by equivocal guanylation of the appropriate hydrazine,[3] confirmed the assignment of the structure IV on the following grounds.

(i) Derivatives.—In contrast to V, which readily gave a benzal derivative, IVa was recovered unchanged after prolonged treatment with benzaldehyde.

(ii) Degradation.—Treatment of IVb with Raney nickel gave 2-(2,6-dimethylphenoxy)ethylamine and guanidine sulfate. Similar treatment of IVa and V did not yield pure products due to partial loss of chlorine. However, in the former case, some guanidine sulfate was isolated, and in the latter ammonia was detected.

(1) Presented in part before the Division of Medicinal Chemistry, 9th National Medicinal Chemistry Symposium of the American Chemical Society, Minneapolis, Minn., June 21–24, 1964.

(2) (a) J. E. Robertson, J. H. Biel, and F. DiPierro, *J. Med. Chem.*, **6**, 381 (1963); (b) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *ibid.*, **6**, 283 (1963); (c) J. H. Short, U. Biermacher, D. A. Dunnigan, and T. D. Leth, *ibid.*, **6**, 275 (1963); (d) C. Cipens and V. Grinsteins, *Zh. Obshch. Khim.*, **32**, 3811 (1962); (e) A. H. Greer and G. B. L. Smith, *J. Am. Chem. Soc.*, **72**, 874 (1950).

(3) Pfizer Ltd., Belgian Patent 629,613 (Oct. 2, 1963); *Chem. Abstr.*, **60**, 14437 (1964). During the preparation of guanoclor on a multi-kilogram scale, it was apparent from infrared spectroscopy that later fractions did contain traces of the isomer V, and a small quantity of the isomer was isolated by repeated fractional crystallization.

(4) *Brit. Med. J.*, **1**, 621 (1964); marketed in Great Britain as Vatensol®.

(5) J. Augstein and S. M. Green, *Nature*, **201**, 628 (1964); T. D. V. Lawrie, A. R. Lorimer, S. G. McAlpine, and H. Reinert, *Brit. Med. J.*, **1**, 402 (1964).

(iii) Spectroscopy.—The n.m.r. spectra of IVa and V were obtained in trifluoroacetic acid and 90% sulfuric acid, conditions which might be expected to mono- and diprotonate, respectively, the aminoguanidine group.[6] The chemical shift of the methylene protons adjacent to the basic group in structures of type III would be expected to move downfield on changing from the mono- to the diprotonated state, as initially the positive charge will be confined mainly to the guanidine group, while on further protonation, a formal positive charge will now be on the adjacent nitrogen. In an isomer of type II, the addition of a second formal charge will make relatively little difference to the deshielding of the α-methylene group, as even in the monoprotonated state this methylene group is adjacent to an atom carrying a partial positive charge.

This prediction is borne out by the results in Table I, in which the α-protons of IVa, in contrast to those in V, experience a considerable change in chemical shift on increasing the acidity of the medium.

TABLE I

CHEMICAL SHIFTS (τ) OF METHYLENE PROTONS ($ArOCH_2CH_2N$–)[a] IN TWO SOLVENTS

	CF_3CO_2H		H_2SO_4	
Compd.	α	β	α	β
IVa	6.52[b]	5.58[b]	*ca.* 5.8[c]	*ca.* 5.5[c]
V	*ca.* 5.8[c]	*ca.* 5.7[c]	*ca.* 5.5[d]	

[a] α and β refer to relative position to the nitrogen atom. [b] Triplet, J = 5 c.p.s. [c] Overlapping complex multiplets. [d] Broad singlet.

(iv) Synthesis.—The N-benzyl-2-(2,6-dimethylphenoxy)ethylamine intermediate VI was nitrosated and reduced with lithium aluminum hydride to give the hydrazine VII. This was treated with 1-amidino-3,5-dimethylpyrazole sulfate and catalytically hydrogenated to IVb, identical with the material obtained directly from the reaction of I with 2-(2,6-dimethylphenoxy)-ethylhydrazine. Application of the reductive alkylation procedure of Finnegan, *et al.*,[7] to 2,6-dimethylphenoxyacetaldehyde and aminoguanidine failed to yield a pure product.

$$ArO(CH_2)_2NHCH_2C_6H_5 \xrightarrow[LiAlH_4]{HNO_2} ArO(CH_2)_2N(NH_2)CH_2C_6H_5$$

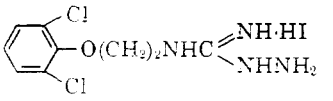
VI VII

The isomer V was synthesized by the following unequivocal method, albeit in poor yield. 2-(2,6-Dichlorophenoxy)ethylhydrazine was treated with cyanogen bromide in aqueous ethanol to give a mixture of the cyanohydrazines VIII and IX. The alkali-soluble derivative VIII was removed from the reaction mixture by extraction with dilute sodium hydroxide, and the remaining IX was treated with ammonia and ammonium sulfate to give V. This compound was distinct from IVa in its infrared spectroscopic and chromatographic characteristics.

$ArO(CH_2)_2NHNHCN$ VIII $\qquad$ $ArO(CH_2)_2N(NH_2)CN$ IX

The above analytical and synthetic methods should be of general application in the field of substituted aminoguanidines.

It is of interest to compare the biological properties of V and the third isomer X,[8] with those of guanoclor (IVa). The powerful adrenergic neurone blocking property of IVa is reduced in the case of V, with respect to both potency and duration, while X proved to be



X

inactive. On the other hand, X was equiactive with isomer IVa as an inhibitor of dopamine β-oxidase *in vitro*, while isomer V possessed only slight activity, as can be seen from Table II.

TABLE II

PER CENT INHIBITION OF DOPAMINE β-OXIDASE *in Vitro*

	Concn., g.-equiv./l.	
Compd.	5×10^{-4}	10^{-4}
IVa	81	67
V	18	0
X	83	68

Experimental[9]

2-(2,6-Dichlorophenoxy)ethylhydrazine.[3]—2-(2,6-Dichlorophenoxy)ethyl bromide[10] (20 g., 0.074 mole) in ethanol (75 ml.) was added slowly to hydrazine hydrate (37 g., 0.74 mole) in ethanol (25 ml.), and the mixture was heated under reflux for 16 hr. The solvent and excess hydrazine were removed under reduced pressure, and the residue was treated with 50% aqueous NaOH. The product, obtained in 80% yield by extraction with chloroform and distillation [b.p. 132–140° (1 mm.), $n^{24}D$ 1.5665], was somewhat unstable but could be stored in the form of the **hydrochloride,** m.p. 110–111° (from methanol–ether).

Anal. Calcd. for $C_8H_{11}Cl_3N_2O$: C, 37.31; H, 4.30; N, 10.87. Found: C, 37.44; H, 4.05; N, 11.00.

2-(2,6-Dimethylphenoxy)ethylhydrazine[3] was prepared in an identical manner from 2-(2,6-dimethylphenoxy)ethyl bromide[11] in 61–80% yield. It had b.p. 112–120° (0.7 mm.), $n^{23}D$ 1.5340.

2-(2,6-Dichlorophenoxy)ethylaminoguanidine Sulfate (IVa).[3]—S-Methylisothiourea sulfate (14.46 g., 0.052 mole) and 2-(2,6-dichlorophenoxy)ethylhydrazine (23.0 g., 0.104 mole) in water (150 ml.) were heated under reflux for 4 hr. The product (14.5 g., 45%) crystallized from the cooled reaction mixture, and, after recrystallization from water, it had m.p. 210–214°. ν_{max} 1675 and 1630 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{26}Cl_4N_8O_6S$: C, 34.62; H, 4.20; N, 17.95; S, 5.12. Found: C, 34.60; H, 4.20; N, 17.69; S, 4.98.

2-(2,6-Dimethylphenoxy)ethylaminoguanidine Sulfate (IVb).[3]—In the same manner, 2-(2,6-dimethylphenoxy)ethylhydrazine was treated with S-methylisothiourea sulfate to give IVb, m.p. 214–216° (from aqueous ethanol), ν_{max} 1680 and 1645 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{38}N_8O_6S$: C, 48.67; H, 7.06; N, 20.65; S, 5.91. Found: C, 48.63; H, 7.18; N, 20.94; S, 5.89.

N-Benzyl-N-nitroso-2-(2,6-dimethylphenoxy)ethylamine.—N-Benzyl-2-(2,6-dimethylphenoxy)ethylamine (VI) (94 g., 0.368 mole) [b.p. 140° (0.6 mm.), $n^{25}D$ 1.5518, prepared by heating 2-(2,6-dimethylphenoxy)ethyl bromide[11] and excess benzylamine at 150° for 3 hr.] was added to 1 *N* HCl (369 ml.) with vigorous stirring. Benzene (50 ml.) was added, and the resulting suspen-

(6) J. J. Pitha, H. Hughes, and G. B. L. Smith, *J. Am. Chem. Soc.*, **70**, 2823 (1948).

(7) W. G. Finnegan, R. A. Henry, and G. B. L. Smith, *ibid.*, **74**, 2981 (1952).

(8) This compound is related to those disclosed by Smith, Kline and French Laboratories, Inc., U. S. Patent 3,131,218 (April 28, 1964).

(9) Melting points were taken on a Kofler hot stage and are corrected. Infrared spectra were run on a Perkin-Elmer Infracord 137 instrument, solids as Nujol mulls, liquids as thin films. Nuclear magnetic resonance spectra were run on a Perkin-Elmer 40-Mc. instrument.

(10) Burroughs Wellcome & Co., U. S. Patent 3,099,599 (July 30, 1963); *Chem. Abstr.*, **60**, 2824 (1964).

(11) P. Hey and G. L. Wiley, *Brit. J. Pharmacol.*, **9**, 471 (1954).

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.

Acknowledgment.—We wish to acknowledge the cooperation of our colleagues in the Pharmacology Department at Pfizer Ltd., who supplied us with the above biological results, and to thank Mr. P. R. Wood for the microanalyses and Mr. J. A. Davidson for his competent assistance.

(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.

(1) This work was supported by U. S. Public Health Service Research Grants CA-03717-05-06 and -07 from the National Cancer Institute.

(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).