Experimental Section⁷

1-Bromo-2-nitro-9-oxofluorene (4).—1-Bromofluoren-2-amine⁴ (15 g, 0.057 mole) was added in small amounts to rapidly stirred cold 40% AcO₂H (300 ml) over a period of 20 min. The resulting suspension was slowly heated (*caution*!) to refluxing, refluxed continuously with stirring for 3 hr, and cooled. After water dilution the precipitate was dissolved in boiling AcOH. To the stirred boiling solution Na₂Cr₂O₇·2H₂O (50 g) was added portionwise within 15 min. Boiling was continued for 30 min and the mixture was cooled. The product was triturated in H₂O and isolated giving 8.5 g (49%). Sublimation at 145–155° (bath) (0.01 mm) gave a sample melting at 221–222°. Anal. (C₁₃H₆BrNO₃) C, H, Br, N.

1-Methylthio-9-oxofluoren-2-amine (5).—To a stirred suspension of 4 (1.4 g) in DMSO (75 ml) a fresh¹⁰ 10% solution of NaSCH₃ in absolute EtOH (3.4 ml, 1 equiv) was added dropwise over a 45-min period. The mixture was continuously stirred at ambient temperature for 46 hr then poured into water containing a few milliliters of concentrated HCl. The product, **1-methyl-thio-2-nitro-9-oxofluorene**, was chromatographed in C₆H₆ (alumina), giving 0.65 g, mp 155–159°. Reduction with SnCl₂·2H₂O (6 g) and concentrated HCl (30 ml) gave a product which was chromatographed twice (alumina, C₆H₆) giving 0.3 g of 5, mp 171–172°. Anal. (C₁₄H₁₁NOS) N, S.

1-Methylthiofluoren-2-amine (6).—A mixture of 5 (0.24 g, 1 mmole), 99–100% hydrazine hydrate (2 ml), KOH (0.5 g), and 2,2'-oxydiethanol (25 ml) was gently refluxed for 0.5 hr, diluted (H₂O), and refrigerated overnight. The product was isolated giving 0.2 g (89%), mp 78–79°. Anal. (C1₄H₁₈NS) N. N-2-(1-Methylthiofluorenyl)acetamide (1).—Acetylation of 6

N-2-(1-Methylthiofluorenyl)acetamide (1).—Acetylation of **6** in AcOH with Ac₂O gave the product, mp 175–176°. Anal. (C₁₆H₁₈-NOS) C, H, N, S.

5-Methylthio-9-oxofluoren-2-amine (7).-2-Nitro-9-oxofluorene-5-amine⁵ (7.5 g) was diazotized in 32% H₂SO₄ (150 ml) at 5-10° with NaNO₂ (3.5 g); 50% HBF₄ (50 ml) was then added and the diazonium fluoroborate was collected, dried, and added in one portion to a stirred solution of potassium ethyl xanthate (50 g)in H_2O (100 ml). The mixture was heated gradually, with stirring, to 100° and cooled. The organic material was extracted (C_6H_6) , washed (H_2O) , dried (Drierite), and evaporated to a solid mass. This was mixed with a hot solution of KOH (1.7 g)in 95% EtOH (100 ml) and stirred at ambient temperature for 27 hr then filtered into 3 N HCl (1 l.). The solid residue was extracted three times with a hot solution of KOH (3 g) in 95% EtOH (50 ml) and filtered into the same 3 N HCl. The precipitated 5-mercapto-2-nitro-9-oxofluorene, mp 123-126°, 3.6 g, was mixed with NaOH (2 g), H₂O (30 ml), and Me₂SO₄ (1.9 g). The mixture was shaken for 10 min and refluxed for 3 hr. After water dilution the methylthic derivative was mixed with SnCl₂. $2H_2O$ (20 g) and concentrated HCl (80 ml). The mixture was boiled with stirring for 45 min and poured into 2 N NaOH (0.5 l.). The crude product (1.3 g) was chromatographed through alumina (C_6H_6) giving pure 7 as deep purple crystals, mp 124-126°. Anal. (C₁₄H₁₁NOS) C, H, N.

5-Methylthiofluoren-2-amine (8).—A mixture of **7** (0.15 g), 99–100% hydrazine hydrate (1 ml), KOH (0.15 g), and 2,2'-oxydiethanol (5 ml) was gently refluxed for 1 hr and diluted (H₂O). The oily precipitate, after refrigeration, was separated and recrystallized from 95% EtOH giving 0.1 g, mp 124–125°. Anal. (C₁₄H₁₃NS) N, S.

N-2-(5-Methylthiofluorenyl)acetamide (2).—Acetylation of 8 with Ac₂O in AcOH gave the product, mp 174–175.5°. Anal. ($C_{16}H_{16}NOS$) C, H, N, S.

N-2-(7-Methylthiofluorenyl)acetamide (3).—Diazotization of 5 g of N-2-(7-aminofluorenyl)acetamide⁶ in 50% HBF₄ (20 ml) and DMSO (10 ml) with NaNO₂ (2.5 g) at <0° gave the diazonium fluoroborate, 7 g (98%), mp 148–150° dec. A slurry of the diazonium salt in H₂O (20 ml) was added with stirring to a solution of potassium ethyl xanthate (10 g) in H₂O (20 ml) at room temperature. The reaction mixture was gradually heated to 80° and cooled. The brown precipitate was collected, mixed with EtOH (30 ml) and an aqueous solution of KOH (3 g), boiled for 1 min, and cooled. MeI (5 ml) was added and the mixture was

heated on a steam bath for 3 min and cooled. The precipitate was filtered giving 4.6 g (82%), mp 206–210°. This was dissolved (Me₂CO) and chromatographed (Me₂CO, acid-washed alumina); evaporation gave 3.4 g, mp 209–210°. *Anal.* (C₁₆H₁₅NOS) C, H, N, S.

N, Ŝ. **7-Methylthiofluoren-2-amine** (9).—A mixture of 3 (2 g), 95%EtOH (150 ml), and concentrated HCl (10 ml) was refluxed for 7 hr and then boiled with the condenser removed until a precipitate started to form. It was cooled, and the precipitate was collected, mixed with H₂O (50 ml), and basified with concentrated NH₄OH. The product was recrystallized from 95% EtOH (Darco) giving 1.3 g (77%), mp 156-157°. *Anal.* (C₁₄H₁₃NS) C, H, N, S.

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2,4-Bis(aryloxy)pyrimidines as Antimicrobial Agents

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In our previous communications¹ it has been shown that 2,4-bis(arylamino)pyrimidines are potent antimicrobial agents. Encouraged by these findings we have prepared a series of 2,4-bis(aryloxy)pyrimidines (1). In this note the syntheses of I, $R_1 = H$ or CH_3 , by condensation of 2,4-dichloro-^{2a} or 2,4-dichloro-5-methylpyrimidine^{2b} with the appropriate phenolic compounds in the presence of anhydrous K_2CO_3 according to the method of Matsukawa and Shirakawa³ are reported. These compounds have been tested against gram-positive and gram-negative bacteria and also against a pathogenic strain of yeast.



The 5-methylbis(arylamino)-^{1d} and 5-unsubstituted bis(arylamino)pyrimidines^{1c} were found to be much more active than the corresponding bisaryloxypyrimidines. In contrast to bisarylaminopyrimidines, the biological activity of the bisaryloxypyrimidines is almost independent of the nature of the substituent in the phenyl ring. Methyl substitution in the 5 position of the pyrimidine ring does not alter significantly the inhibitory activity.

Experimental Section

General Method of Synthesis of 2,4-Bis(aryloxy)pyrimidines. --2,4-Dichloropyrimidine (0.01 mole) and phenol or substituted phenol (0.025 mole) were mixed as a melt and subsequently

⁽⁷⁾ All melting points were taken on a Fisher-Johns block and are corrected to standards. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within $\pm 0.4\%$ of the theoretical values. Ir spectra (KBr) (Beckman IR-5) were as expected. Analyses were done by Schwarzkopf Laboratories, Woodside, N. Y., and by A. Bernhardt, Mülheim (Ruhr).

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Notes

TABLE 1 2,4-Bis(Aryloxy)pyrimidines

Aro N OAr

Compd	R	Δr	Yield, % (erude)	. – Rea Time, min	etion″. Temp, °C	$M_{\rm Pe} \approx C^2$	$\frac{\text{Solvent of}}{\text{recrystn}^d}$	Formula
I	H	$C_6 \Pi_5$	95	30	120	111	H	$\mathrm{C}_{16}\mathrm{H}_{12}\mathrm{N}_{2}\mathrm{O}_{2}$
П	H	p-CH ₃ C ₆ H ₄	95	30	120	$105 \cdot 107$	11	$C_{18}H_{16}N_2O_2$
Ш	H	p-OCH ₃ C ₆ H ₄	98	30	120	116-117	H - E	$C_{18}H_{16}N_2O_4$
I V	Н	p-ClC ₆ H ₄	98	60	130	117-118	Н	$\mathrm{C}_{16}\mathrm{H}_{10}\mathrm{Cl}_2\mathrm{N}_2\mathrm{O}_2$
V	H	ρ -BrC ₆ H ₄	97	60	130	140-141	ΗE	$\mathrm{C}_{16}\mathrm{H}_{10}\mathrm{Br}_{2}\mathrm{N}_{2}\mathrm{O}_{2}$
VI	H	$p-\mathrm{NO}_2\mathrm{C}_6\mathrm{H}_4$	90	60	160	162 - 163	А	$\mathrm{C}_{16}\mathrm{H}_{10}\mathrm{N}_4\mathrm{O}_6$
VП	CH_3	$C_6 \Pi_0$	98	30	120	85-87	11	$\mathrm{C}_{17}\mathrm{N}_{14}\mathrm{N}_{2}\mathrm{O}_{2}$
VIII	CH_3	p-CH ₃ C ₆ H ₄	90	30	120	107-110	H	$\mathrm{C}_{19}\mathrm{H}_{18}\mathrm{N}_{2}\mathrm{O}_{3}$
IX	CH_3	p-OCH ₃ C ₆ H ₄	90	30	120	80	H	$\mathrm{C}_{19}\mathrm{H}_{18}\mathrm{N}_{2}\mathrm{O}_{4}$
Х	CH_3	p-ClC ₆ H ₄	90	60	630	115-117	11	$\mathrm{C}_{17}\mathrm{H}_{12}\mathrm{Cl}_2\mathrm{N}_2\mathrm{O}_2$
XI	CH_3	p-BrC ₆ H ₄	97	60	130	108 - 110	$\mathbf{H} \rightarrow \mathbf{E}$	$\mathrm{C}_{17}\mathrm{H}_{12}\mathrm{Br}_{2}\mathrm{N}_{2}\mathrm{O}_{2}$
XП	CH_3	p-NO ₂ C ₆ H ₄	90	60	160	132 - 135	.\	$\mathrm{C}_{17}\mathrm{H}_{12}\mathrm{N}_{4}\mathrm{O}_{6}$

^{*a*} In the syntheses of compounds VI and XII, 5 ml of toluene was added to the reaction mixture. ^{*b*} All melting points were determined in capillary tubes in Gallen-Kumph apparatus and are corrected. ^{*c*} All compounds were analyzed for C, H, N. Analytical data were within $\pm 0.4\%$ of the theoretical values. ^{*d*} H = hexane, E = Et₂O, A = EtOH.

TABLE II ANTIMICROBIAL ACTIVITIES OF 2,4-BIS(ARYLOXY)PYRIMIDINES

	Copen for 50% inhib of growth up tol				
Compd	$S.\ faecalis$	8. typhimurium	C. albicans	$E, \ coli$ B	
I	18.40	7.20	24,00	8.20	
II	5,80	6.40	16.80	6.50	
III	16.50	8.21	16.40	7.00	
IV	5,60	5,60	14.80	5.00	
V	7.60	9.20	23.60	10.40	
VI	5.40	6.60	14.16	5.80	
VII	20,80	9,00	21,60	8.30	
VIII	6.20	7.00	18.00	7.20	
IX	11.60	8,50	15,80	7,60	
X	5.80	6,60	16.80	5,60	
XI	7.80	9,80	22.80	11.00	
XII	5.20	8.50	13.20	5,80	
2,4-Bis(p-chloroanilino)pyrimidine	0.80	0.36	0.62	0.60	
2,4-Bis $(p$ -chloroanilino)-5-methylpyrimidine	1.30	0.85	0.92	1.0	
Neomycin	a	1.55	1.10	1.30	
Chloramphenicol	1,50	0.66	(1	1,00	

" Little or no activity.

cooled to room temperature. Finely powdered anhydrous K_2CO_3 (0.025 mole) was added to the reactants and mixed well. The mixture was heated on an oil bath at the optimum reaction temperature until completion of the reaction (see Table I) and cooled, and on addition of 5% KOH (20 ml) an oily substance separated out. The oil was extracted with hexane-ether, washed (dilute KOH, H₂O), and dried (Na₂SO₄). Crystals appeared on evaporating the solvent.

Inhibition of Growth of Microorganisms.—All compounds including two highly active 2,4-bis(arylamino)pyrimidine derivatives^{1e,d} and two well-known broad-spectrum antibiotics were tested for antimicrobial activity against *Streptococcus faecalis*, *Salmonella typhimurium*, *Escherichia coli* B, and a pathogenic strain of yeast, *Candida albicans*. The concentrations of these compounds necessary for 50% inhibition of growth were determined turbidimetrically by serial dilution technique in test tubes using liquid growth medium^{1b} (see Table II).

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Folic Acid Analogs. I. p-{[(2,4-Diamino-5-pyrimidinyl)methyl]amino}benzoylL-glutamic Acid and Related Compounds^{1,2}

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A number of folic acid antagonists owe their effectiveness to the inhibition of dihydrofolate reductase and thymidylate synthetase. The former enzyme is necessary for the reduction of folic acid (FA) to dihydrofolic acid (FAH₂) and then to tetrahydrofolic acid (FAH₁), and the latter is responsible, together with thymidine kinase, for cellular synthesis of thymidylic acid. In-

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⁽²⁾ Presented in part before the Division of Medicinal Chemistry, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, Abstract N-67.