Experimental Section[7]

1-Bromo-2-nitro-9-oxofluorene (4).—1-Bromofluoren-2-amine[4] (15 g, 0.057 mole) was added in small amounts to rapidly stirred cold 40% AcO_2H (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 $Na_2Cr_2O_7 \cdot 2H_2O$ (50 g) was added portionwise within 15 min. Boiling was continued for 30 min and the mixture was cooled. The product was triturated in H_2O and isolated giving 8.5 g (49%). Sublimation at 145–155° (bath) (0.01 mm) gave a sample melting at 221–222°. *Anal.* ($C_{13}H_6BrNO_3$) 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 fresh[1c] 10% solution of $NaSCH_3$ 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-methylthio-2-nitro-9-oxofluorene,** was chromatographed in C_6H_6 (alumina), giving 0.65 g, mp 155–159°. Reduction with $SnCl_2 \cdot 2H_2O$ (6 g) and concentrated HCl (30 ml) gave a product which was chromatographed twice (alumina, C_6H_6) giving 0.3 g of **5**, mp 171–172°. *Anal.* ($C_{14}H_{11}NOS$) 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 (H_2O), and refrigerated overnight. The product was isolated giving 0.2 g (89%), mp 78–79°. *Anal.* ($C_{14}H_{13}NS$) N.

N-2-(1-Methylthiofluorenyl)acetamide (1).—Acetylation of **6** in AcOH with Ac_2O gave the product, mp 175–176°. *Anal.* ($C_{16}H_{15}NOS$) C, H, N, S.

5-Methylthio-9-oxofluoren-2-amine (7).—2-Nitro-9-oxofluorene-5-amine[5] (7.5 g) was diazotized in 32% H_2SO_4 (150 ml) at 5–10° with $NaNO_2$ (3.5 g); 50% HBF_4 (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), H_2O (30 ml), and Me_2SO_4 (1.9 g). The mixture was shaken for 10 min and refluxed for 3 hr. After water dilution the methylthio derivative was mixed with $SnCl_2 \cdot 2H_2O$ (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.* ($C_{14}H_{11}NOS$) 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 (H_2O). The oily precipitate, after refrigeration, was separated and recrystallized from 95% EtOH giving 0.1 g, mp 124–125°. *Anal.* ($C_{14}H_{13}NS$) N, S.

N-2-(5-Methylthiofluorenyl)acetamide (2).—Acetylation of **8** with Ac_2O in AcOH gave the product, mp 174–175.5°. *Anal.* ($C_{16}H_{15}NOS$) C, H, N, S.

N-2-(7-Methylthiofluorenyl)acetamide (3).—Diazotization of 5 g of N-2-(7-aminofluorenyl)acetamide[6] in 50% HBF_4 (20 ml) and DMSO (10 ml) with $NaNO_2$ (2.5 g) at <0° gave the diazonium fluoroborate, 7 g (98%), mp 148–150° dec. A slurry of the diazonium salt in H_2O (20 ml) was added with stirring to a solution of potassium ethyl xanthate (10 g) in H_2O (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 (Me_2CO) and chromatographed (Me_2CO, acid-washed alumina); evaporation gave 3.4 g, mp 209–210°. *Anal.* ($C_{16}H_{15}NOS$) C, H, N, S.

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 H_2O (50 ml), and basified with concentrated NH_4OH. The product was recrystallized from 95% EtOH (Darco) giving 1.3 g (77%), mp 156–157°. *Anal.* ($C_{14}H_{13}NS$) C, H, N, S.

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(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 ±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).

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

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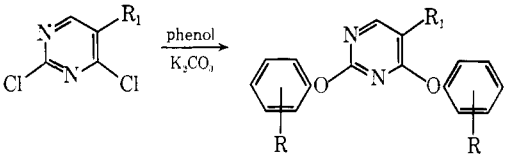
In our previous communications[1] it has been shown that 2,4-bis(arylamino)pyrimidines are potent antimicrobial agents. Encouraged by these findings we have prepared a series of 2,4-bis(aryloxy)pyrimidines (**1**). In this note the syntheses of I, R_1 = H or CH_3, by condensation of 2,4-dichloro-[2a] 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 Shirakawa[3] are reported. These compounds have been tested against gram-positive and gram-negative bacteria and also against a pathogenic strain of yeast.



1

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

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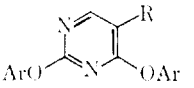
(2) (a) E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 1152 (1930); (b) O. Gerngross, *Ber.*, **38**, 3408 (1905).

(3) T. Matsukawa and K. Shirakawa, *J. Pharm. Soc. Japan*, **71**, 1313 (1951).

TABLE I

2,4-BIS(ARYLOXY)PYRIMIDINES



Compd	R	Ar	Yield, % recrystd	Reaction[a] Time, min	Reaction[a] Temp, °C	Mp, °C[b]	Solvent of recrystn[d]	Formula[c]
I	H	C_6H_5	95	30	120	111	H	$C_{16}H_{12}N_2O_2$
II	H	p-$CH_3C_6H_4$	95	30	120	105–107	H	$C_{18}H_{16}N_2O_2$
III	H	p-$OCH_3C_6H_4$	98	30	120	116–117	H–E	$C_{18}H_{16}N_2O_4$
IV	H	p-ClC_6H_4	98	60	130	117–118	H	$C_{16}H_{10}Cl_2N_2O_2$
V	H	p-BrC_6H_4	97	60	130	140–141	H–E	$C_{16}H_{10}Br_2N_2O_2$
VI	H	p-$NO_2C_6H_4$	90	60	160	162–165	A	$C_{16}H_{10}N_4O_6$
VII	CH_3	C_6H_5	98	30	120	85–87	H	$C_{17}N_{14}N_2O_2$
VIII	CH_3	p-$CH_3C_6H_4$	90	30	120	107–110	H	$C_{19}H_{18}N_2O_2$
IX	CH_3	p-$OCH_3C_6H_4$	90	30	120	89	H	$C_{19}H_{18}N_2O_4$
X	CH_3	p-ClC_6H_4	90	60	130	115–117	H	$C_{17}H_{12}Cl_2N_2O_2$
XI	CH_3	p-BrC_6H_4	97	60	130	108–110	H–E	$C_{17}H_{12}Br_2N_2O_2$
XII	CH_3	p-$NO_2C_6H_4$	90	60	160	132–135	A	$C_{17}H_{12}N_4O_6$

[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 ±0.4% of the theoretical values. [d] H = hexane, E = Et_2O, A = EtOH.

TABLE II

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

Compd	Conen for 50% inhib of growth, μg/ml *S. faecalis*	*S. 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	a	1.00

[a] 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, H_2O), and dried (Na_2SO_4). Crystals appeared on evaporating the solvent.

Inhibition of Growth of Microorganisms.—All compounds including two highly active 2,4-bis(arylamino)pyrimidine derivatives[1c,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}benzoyl-L-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 (FAH_2) and then to tetrahydrofolic acid (FAH_4), and the latter is responsible, together with thymidine kinase, for cellular synthesis of thymidylic acid. In-

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