to observe possible drug activity against a wide spectrum of different types of organisms. The test organisms included were gram-positive human pathogens, gram-negative human pathogens, acid-fast bacteria (representing the leprosy-tuberculosis group), common yeasts, and pathogenic fungi. The results are recorded in Table III.

TABLE III

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY[a]

Compd	Acid-fast bacteria	Gram-positive bacteria	Gram-negative bacteria	Fungi and yeasts
1	−	−	−	−
2	−	−	−	+
3	−	−	−	−
4	−	+	−	−
6	−	−	−	+

[a] + = inhibition, − = no inhibition.

Experimental Section[b]

N-3-Azabicyclo[3.2.2]nonylmethyl-5-methylisatin (II).—A solution of 3-azabicyclo[3.2.2]nonane (3.12 g, 0.25 mole) in 10 ml of ethanol was added to a slurry consisting of 5-methylisatin (4.02 g, 0.25 mole), 2.5 ml of 37% formalin, and 10 ml of ethanol with shaking. The resulting reaction mixture was stirred for 30 min at room temperature and then warmed for another 30 min on a steam bath. The contents of the flask, upon refrigeration overnight, gave a product melting at 160–162°. An analytical sample was prepared by three successive crystallizations from ethanol; mp 162–164°, yield 3.5 g (47%), ν_{max} 1724 cm^{-1} (C=O).

Anal. Calcd for $C_{18}H_{22}N_2O_2$: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.33; H, 7.45; N, 9.41.

The nmr spectrum of II is consistent with the proposed structure. The spectrum showed a singlet at δ 1.66 attributed to the ten protons of the cyclohexane ring and a doublet at 2.73–2.8 which is attributed to the CH_2 protons (4 H) adjacent to N. A sharp singlet was observed at δ 4.5. This latter peak has been assigned to the two CH_2 protons between the two N atoms. A singlet at δ 2.36 was due to CH_3 at position 5 and a complex pattern at δ 6.96–7.51 to the protons (3 H) of the aromatic ring.

N-Dialkylaminomethylisatin β-Thiosemicarbazones (Table I). Method A.—To a solution of 0.01 mole of isatin N-Mannich base in 15 ml of absolute ethanol was added, in one portion, 0.91 g (0.01 mole) of thiosemicarbazide. The reaction mixture was stirred overnight at room temperature. The resulting solid was collected by filtration and washed with absolute ethanol. An analytical sample was prepared by repeated crystallizations from ethyl acetate.

Method B.—Thiosemicarbazide (0.91 g, 0.01 mole) was dissolved in 10 ml of distilled water by warming on a water bath. To this solution there was added the isatin N-Mannich base (0.01 mole) followed by 10 ml of ethanol. The reaction mixture was heated under reflux for 1.5 hr. At the end of this time the contents of the flask were cooled and the product was collected by filtration; the product was then washed with ethanol. For characterization an analytical sample was prepared by crystallization from ethyl acetate.

Acknowledgments.—This investigation was supported in part by Grant No. AI 04701 from the National Institute of Allergy and Infectious Diseases, National Institute of Health, U. S. Public Health Service, Bethesda, Md. The authors are grateful to Dr. Lyman Magee and Mr. William Bing for biological data and to Dr. R. L. Settine for nmr data.

(2) All melting points were taken in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. The infrared spectra were determined in Nujol mull on a Perkin-Elmer Model 137 spectrophotometer. Nmr spectrum was taken on a Varian-A-60 spectrometer in $CDCl_3$ using Me_4Si as an internal standard. Microanalyses were done by Dr. Alfred Bernhardt, Mülheim (Ruhr), Germany, and Galbraith Laboratories, Knoxville, Tenn., or through the courtesy of Dr. Paul Craig, Smith Kline and French Laboratories, Philadelphia, Pa.

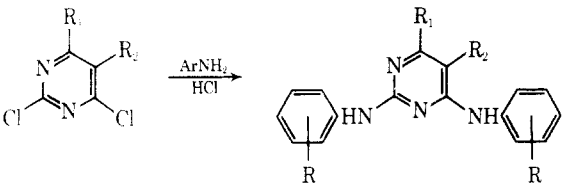
2,4-Bis(arylamino)-5-methylpyrimidines as Antimicrobial Agents

DOLLY GHOSH AND MINA MUKHERJEE

Biochemistry Unit, Department of Chemistry, Bose Institute, Calcutta 9, India

Received April 17, 1967

In our previous communications[1] it has been shown that 2,4-bis(arylamino)pyrimidines (A, R_1 = OH; R_2 = H and R_1 = R_2 = H) possess potent antimicrobial activities. The biological activities of these compounds have not been studied in detail so far. We have been encouraged to study 2,4-bis(arylamino)pyrimidines in some detail since some compounds of this series showed high antifungal activity.[1c] In this communication, we wish to report the synthesis and growth inhibitory activity of a number of compounds related to type A, where R_1 = H and R_2 = CH_3. These compounds have been tested against gram-positive and gram-negative bacteria and also a pathogenic strain of yeast. The growth inhibitory activity of these synthetic pyrimidines has been compared with that of neomycin (a known antifungal agent), chloramphenicol, and 6-azauracil.



A

The 2,4-bis(arylamino)-5-methylpyrimidines were synthesized by the acid-catalyzed condensation of 2,4-dichloro-5-methylpyrimidine[2] with appropriate aromatic amines.

In general, it has been observed that the antimicrobial activity of 2,4-bis(arylamino)-5-methylpyrimidines is enhanced when groups with positive σ constants are substituted in the phenyl ring, whereas groups with negative σ constants decrease the activity considerably. Thus chloro-substituted derivatives (III–V) show maximum activity, whereas minimum activity is exhibited by the hydroxy-substituted compound VI. Similar biological activities are shown by 2,4-bis(arylamino)-pyrimidines and 2,4-bis(arylamino)-6-hydroxypyrimidines which have been reported earlier.[1b,c]

Studies on the mechanism of inhibition of these active compounds are in progress and will be reported elsewhere.

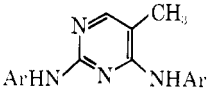
Experimental Section

2,4-Bis(*p*-chloroanilino)-5-methylpyrimidine (III).—2,4-Dichloro-5-methylpyrimidine (1.63 g, 0.01 mole) was added to a warm solution of *p*-chloroaniline (3.8 g, 0.03 mole) in 2.7 ml of concentrated HCl in 20 ml of water, and refluxed on a sand bath.

(1) (a) D. Roy, S. Ghosh, and B. C. Guha, *J. Org. Chem.*, **25**, 1909 (1960); (b) D. Roy, S. Ghosh, and B. C. Guha, *Arch. Biochem. Biophys.*, **92**, 366 (1961); (c) D. Ghosh, *J. Med. Chem.*, **9**, 424 (1966).

(2) O. Gerngross, *Ber.*, **38**, 3408 (1905).

TABLE I
2,4-BIS(ARYLAMINO)-5-METHYLPYRIMIDINES



Compd	Ar	Yield, % (crude)	Reaction time, hr	Mp, °C[a]	Solvent of recrystn	Formula	—% carbon— Calcd	Found	—% hydrogen— Calcd	Found	—% nitrogen— Calcd	Found
I	C_6H_5	82	1.5	175–177	Aq ethanol	$C_{17}H_{16}N_4$	73.91	73.71	5.83	6.00	20.28	20.08
II	p-$NO_2C_6H_4$	85	0.5	>300	DMF	$C_{17}H_{16}N_6O_4$	55.73	55.59	3.82	4.01	22.95	22.78
III	p-ClC_6H_4	92	1	215–217	Aq ethanol	$C_{17}H_{14}Cl_2N_4$	59.15	59.20	4.05	4.15	16.23	16.00
IV	m-ClC_6H_4	86	1	183	Aq ethanol	$C_{17}H_{14}Cl_2N_4$	59.15	58.99	4.05	4.19	16.23	16.43
V	o-ClC_6H_4	80	1	142–143	Aq ethanol	$C_{17}H_{14}Cl_2N_4$	59.15	59.20	4.05	4.30	16.23	15.98
VI	p-OHC_6H_4	77	6	>300	1 N HCl	$C_{17}H_{16}N_4O_2 \cdot HCl$	59.50	59.42	4.60	4.73	16.22	16.00
VII	p-$CH_3C_6H_4$	90	1	170	Aq ethanol	$C_{19}H_{20}N_4$	75.00	75.10	6.57	6.70	18.42	18.36
VIII	p-$OCH_3C_6H_4$	70	2	180–183	Aq ethanol	$C_{19}H_{20}N_4O_2$	67.85	68.10	5.95	5.99	16.60	16.72
IX	p-$COCH_3C_6H_4$	70	3	>300	1 N HCl	$C_{21}H_{20}N_4O_2 \cdot HCl$	60.30	60.22	5.04	5.24	14.19	14.00

[a] All melting points were determined in capillary tubes in a Gallenkumph apparatus and are corrected.

Crystals began to appear within 15–20 min. The refluxing was stopped after 1 hr and the reaction mixture was kept overnight in a refrigerator. The crystalline product was filtered off and washed with cold water. It was then suspended in about 30 ml of water, neutralized with dilute NH_4OH, cooled, and collected by filtration as free base. This compound could be easily recrystallized from 80% ethanol.

The other compounds listed in Table I were synthesized by the same general method. As indicated in Table I the time of refluxing had to be extended in certain cases. Some compounds were recrystallized as hydrochlorides since they could not be satisfactorily crystallized as free bases. All compounds were recrystallized from suitable solvent and were dried *in vacuo* at 110° for 24 hr before analysis.

Inhibition of Growth of Microorganisms.—All compounds were tested for their antimicrobial activity against *Streptococcus faecalis*, *Escherichia coli* B, *Salmonella typhimurium*, and a pathogenic strain of yeast, *Candida albicans*. The concentrations of synthetic compounds necessary for 50% inhibition of growth were determined turbidimetrically by serial dilution technique in test tubes using liquid growth medium[1b] (shown in Table II).

TABLE II
ANTIMICROBIAL ACTIVITIES OF
2,4-BIS(ARYLAMINO)-5-METHYLPYRIMIDINES

Compd	Concn for 50% inhib of growth, μg/ml — *S. faecalis*	*E. coli* B	*S. typhimurium*	*C. albicans*
I	2.15	1.96	1.60	9.00
II	[a]	[a]	[a]	[a]
III	1.30	1.00	0.85	0.92
IV	2.20	1.12	0.95	1.06
V	2.40	1.30	1.00	2.60
VI	81.80	102.00	22.00	51.00
VII	1.80	1.32	0.90	4.30
VIII	2.00	1.80	1.00	9.60
IX	1.95	8.50	2.00	23.50
6-Azauracil	12.00	7.20	5.60	[b]
Neomycin	[b]	1.30	1.55	1.10
Chloramphenicol	1.50	1.00	0.66	[b]

[a] Could not be tested due to low solubility in the common solvents. [b] Little or no activity.

Acknowledgment.—The authors wish to thank Dr. D. M. Bose, Director, Bose Institute, for his interest in this work. Thanks are also due to Dr. A. Sen for helpful suggestions and to Mrs. C. Dutta for microanalyses. One of the authors (M. M.) is a Fellow of Council of Scientific and Industrial Research, Government of India.

Tumor Localizing Agents. II. Radioiodinated Analogs of 1,1-Dichloro-2,2-bis(chlorophenyl)ethane

R. E. COUNSELL, R. E. WILLETTE, AND W. DIGUILIO

College of Pharmacy and Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48104

Received March 15, 1967

Wagner[1] has reviewed the important role currently being played by radioactive pharmaceuticals as diagnostic agents in clinical medicine. With the advent of a number of radiopharmaceuticals and the development of accurate detection instruments, it is now possible to externally scan most organs and major parts of the body. To date, however, no agent has been found which is suitable for photoscanning the adrenal gland and its associated tumors. As part of a broad program aimed at the development of a radiopharmaceutical which may be of value in the diagnosis and therapy of adrenal tumors, we wish to report on the synthesis and tissue distribution of some radioiodinated analogs of 1,1-dichloro-2,2-bis(chlorophenyl)ethane (DDD).

Our interest in structures related to DDD was prompted by the many reports in the literature indicating a predilection of these substances for adrenal tissue.[2] Nelson and Woodard[3] observed that commercially available technical p,p'-DDD caused necrosis of certain regions of the adrenal cortex in dogs. Some years later, however, two groups found that the adrenocorticolytic action of the commercial product was actually due to the o,p' isomer present as a contaminant.[4,5] Since that time, o,p'-DDD has been the subject of a number of biological and clinical investigations and these have been recently reviewed by Nichols.[2] Further stimulus to the study of o,p'-DDD and related compounds was provided when this substance was found to produce tumor regression in cases of metastic adrenal cortical carcinoma[6] and to cause remission of symptoms in patients with Cushing's syndrome.[7]

(1) H. N. Wagner, *Clin. Pharmacol. Therap.*, **4**, 351 (1963).
(2) *Cf.* J. Nichols in "The Adrenal Cortex," H. D. Moon, Ed., P. B. Hoebner, Inc., New York, N. Y., 1961, p 84.
(3) A. A. Nelson and G. Woodard, *Arch. Pathol.*, **48**, 387 (1949).
(4) J. Nichols and G. Hennigar, *Exptl. Med. Surg.*, **15**, 310 (1957).
(5) C. Cueto and J. H. V. Brown, *Endocrinology*, **62**, 324 (1958).