ml. of ether, 3 × 200 ml. of acetone, and 3 × 200 ml. of boiling water. The product was then recrystallized from dimethylformamide and water to yield 27 g. (46%) of 5-(2,4-dinitroanilino)-2,4-pyrimidinediol, as golden needles, m.p. 312–313° dec.; $\lambda_{max}^{pH\ 1}$ 246 mμ (ε 15,800); $\lambda_{shoulder}^{pH\ 1}$ 285 mμ (ε 10,000), 340 mμ (ε 5,800); $\lambda_{max}^{pH\ 11}$ 286 mμ (ε 12,400), 346 mμ (ε 10,000).

Anal. Calcd. for $C_{10}H_7N_5O_6 \cdot H_2O$: C, 38.6; H, 2.9; N, 22.5. Found: C, 38.2; H, 3.1; N, 22.4.

The water of hydration can be removed by drying at 130° in a vacuum oven for 24 hr. (*Anal.* Calcd.: C, 41.0; H, 2.4. Found: C, 41.2; H, 2.8.)

Acknowledgment.—The authors wish to express their appreciation to Mr. Wayne H. Nyberg, Mrs. Margaret L. Rounds, Miss Phyllis G. Shaul, Mrs. Beverly Ann Smith, Mrs. Carol R. Tuttle and Mr. Hal P. Van Fossen for their valuable assistance in performing analytical, instrumental and paper chromatographic measurements.

5-Benzyl-2,4-diaminopyrimidines as Antibacterial Agents. I. Synthesis and Antibacterial Activity *in vitro*

BARBARA ROTH, ELVIRA A. FALCO, AND GEORGE H. HITCHINGS

Burroughs Wellcome and Co. (U. S. A.) Inc.,
The Wellcome Research Laboratories,
Tuckahoe, New York

AND S. R. M. BUSHBY

The Wellcome Research Laboratories,
Beckenham, Kent, England

Received May 31, 1962

A series of 5-benzyl-2,4-diaminopyrimidines has been synthesized and tested for antibacterial activity. Maximal activity occurs among those compounds which are unsubstituted in the pyrimidine 6-position, possess unsubstituted amino groups in the 2- and 4-positions and bear one or more alkoxyl groups in the *meta* and *para* positions of the benzene nucleus. These compounds have high activity against Gram positive microorganisms and significant activity against a variety of Gram negative bacteria. Trimethoprim, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine, has been selected for further study on the basis of the magnitude and breadth of its antibacterial activities.

The discovery that many 5-benzyl-2,4-diaminopyrimidines[1] possess a high degree of antibacterial, as well as antimalarial, activity[1–5] has

led to the synthesis of a large number of additional substituted benzylpyrimidines, with the objective of seeking derivatives with the widest antibacterial spectrum and optimum effectivity, particularly against Gram negative organisms. It is the purpose of this paper to describe the new substituted benzyl derivatives which have been prepared in our laboratories during the years intervening since the original publication[1] by Falco and co-workers in 1951, to show structure–activity relationships among the various 5-benzylpyrimidines synthesized to date and the spectrum of activity of the more active members of the series against bacterial pathogens in *in vitro* tests. Results of *in vivo* tests and biochemical studies will be described in separate communications to follow.

It was observed at a very early date that the 5-benzyl-2,4-diaminopyrimidines which had the highest antimalarial activity contained 6-alkyl groups (optimally methyl) in the pyrimidine ring, and *p*-halo or nitro substituents in the benzene ring.[3] Removal of the 6-alkyl group had a rather remarkable effect on the activities against microorganisms, in that the antimalarial activity was considerably depressed, but antibacterial activity was very markedly increased. Among the early compounds, highest activity against Gram negative organisms, such as *Proteus vulgaris*, was found among those containing *p*- and *m*-methoxyl and halo groups. Attention therefore was focused particularly on the preparation of compounds containing various combinations of alkoxyl and halo groups in the benzene ring, in an effort to find derivatives of minimum toxicity and maximum activity.

The synthetic methods used for the preparation of many of the new pyrimidines were essentially those of Falco and co-workers.[1] Arylcinnamic acids were prepared from the corresponding aldehydes by reaction with malonic acid according to the Doebner reaction.[6] A number of new alkoxybenzaldehydes were prepared for this purpose, using known techniques. The cinnamic acids were esterified, reduced, and formylated with ethyl formate. The resultant crude formyl derivatives were condensed directly with guanidine to produce 2-amino-5-benzyl-4-hydroxypyrimidines, which upon chlorination and amination yielded the 2,4-diamino derivatives. In a few in-

(1) E. A. Falco, S. DuBreuil, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3758 (1951).
(2) E. A. Falco, L. G. Goodwin, G. H. Hitchings, I. M. Rollo, and P. B. Russell, *Brit. J. Pharmacol.*, **6**, 185 (1951).
(3) G. H. Hitchings, *Am. J. Clin. Nutrition*, **3**, 321 (1955).
(4) G. B. Elion, S. Singer, and G. H. Hitchings, *Antibiotics and Chemotherapy*, **10**, 556 (1960).
(5) G. H. Hitchings and S. R. M. Bushby, Vth Internat. Congress of Biochem., Moscow, p. 165 (1961).
(6) O. Doebner, *Ber.*, **33**, 2140 (1900); **35**, 1136 (1902).

stances the 4-chloro compounds reacted with alkylamines to produce the 4-alkylamino-2-amino analogs.

Many of the pyrimidines which contain bromo or nitro groups in the benzene ring were prepared by bromination or nitration of the 5-benzyl-2,4-diaminopyrimidines. In the majority of cases, 3,4-dialkoxybenzyl derivatives were used for this purpose. It was found that 2,4-diamino-5-(3,4-dimethoxybenzyl)-pyrimidine gave 5-(2-bromo-4,5-dimethoxybenzyl)-2,4-diaminopyrimidine exclusively upon bromination. Its structure was proved by independent synthesis, starting from 2-bromo-4,5-dimethoxybenzaldehyde. It was assumed that the related alkoxyl derivatives behaved similarly upon bromination; the structures were not proved.

Since it had not been demonstrated previously that 2,4-diamino substituents were required for activity against microörganisms in the 5-benzylpyrimidine series, a number of pyrimidines were prepared containing mercapto or hydroxy substituents in place of one or both amino groups. These were prepared by condensing the sodium salts of ethyl α-formyl-hydrocinnamates with thiourea in ethanol, which yielded 5-benzyl-4-hydroxy-2-mercaptopyrimidines.[7-9] The 2- and 4-substituents were transformed to methylthio, hydroxy, and amino derivatives by well known techniques which are described in the experimental section.

The *in vitro* antibacterial assays were carried out for the most part by determining the minimum concentration of benzylpyrimidine which was required to inhibit growth in cultures of various organisms in nutrient agar and other media. Assays with *Streptococcus pyogenes* were carried out also in whole blood, because experience has shown that an unknown substance, or substances, is present in blood which reverses the antibacterial activity of many agents which are active in nutrient agar. Although most of the benzylpyrimidines which were active in nutrient agar were also active in whole blood, there were a few cases where the activity was considerably diminished, as seen in Table IX (see, for example, compounds LXIV, XLVIII, and LXXI).

In some cases it was found desirable to have a quick screening procedure available for determination of antibacterial potentialities. For such purposes, an agar plate was employed. Paper discs, impregnated with solutions of the compound, were applied to the plate which had been inoculated with the bacteria. Zones of inhibition then were determined. This method was used chiefly to spot activity

(7) H. L. Wheeler and D. F. McFarland, *Am. Chem. J.*, **42**, 101 (1909).
(8) T. B. Johnson and J. C. Ambelang, *J. Am. Chem. Soc.*, **60**, 2941 (1938).
(9) E. A. Falco, P. B. Russell, and G. H. Hitchings, *ibid.*, **73**, 4466 (1951).

vs. Proteus vulgaris, although other organisms also were tested. Compounds screened by this technique are found in Table XII.

Tables X and XII show the antibacterial activities of benzylpyrimidines other than 2,4-diamino-6-unsubstituted derivatives. It will be seen that the 2,4-diamino-6-methyl derivatives are virtually inactive against *P. vulgaris* and have considerably lower activity *vs. S. aureus* than the corresponding 6-unsubstituted derivatives. Activity *vs. St. pyogenes* is retained, however. Introduction of a 6-hydroxyl substituent almost completely abolishes the activity. Conversion of a 4-amino to a methylamino or other alkylamino group gives products which are devoid of interest as antibacterial agents. A similar result is observed when the 4-amino group is replaced by hydroxyl or chloro. The 4-amino-2-hydroxy, 2,4-dihydroxy, 4-hydroxy-2-mercapto, and 4-amino-2-mercapto pyrimidines are likewise inactive. The interest then focuses solely on 5-benzyl-2,4-diamino-6-unsubstituted pyrimidines.

Data pertaining to the relation between structure and antibacterial activity of derivatives with various substituents in the benzene ring are presented in Tables IX and XII. It is to be noted first that very few of the closely related compounds which are listed here have high activity against *P. vulgaris*, and that variations which may increase the activity against *S. aureus* or *St. pyogenes* often result in a lowering of the activity against *P. vulgaris*. Fortunately the reverse is not necessarily true, for a few of the compounds have activity against a wide variety of microörganisms.

It will be seen that the most active compounds contain *meta* alkoxyl or halo substitution, and preferably 4-alkoxyl or hydroxyl substitution as well. (4-Hydroxyl substitution later was found to be inadvisable in *in vivo* tests, so only one such compound is listed here.) The best 3-alkoxyl substitution seems to be methoxyl. The 3-ethoxyl derivative is inactive *vs. P. vulgaris*, although the 3-methoxyl derivative is very active. In the 4-position, a methoxyl group is best against *P. vulgaris*, but higher alkoxyl groups, including propoxyl, butoxyl, and amyloxyl, but not octyloxyl, give better results against *S. aureus*. This is true with the single exception of the 3,4,5-trimethoxyl derivative (LXV), which is the one compound which stands out for the breadth of its spectrum of activity. In this case, the substitution of a 4-higher-alkoxyl group does not increase the activity against *S. aureus*, and does markedly decrease the activity against *P. vulgaris*. Among other 3,4,5-trisubstituted derivatives, the 3,4-dimethoxy-5-bromo derivative has high broad-spectrum activity, but is a shade inferior to the derivative without the 5-bromo substituent (49–210).

Higher alkoxyl groups (*i.e.*, propoxyl and butoxyl) in the 4-position of this series again have the effect of increasing activity against *S. aureus*, but decreasing activity against *P. vulgaris*. It appears that a *meta* bromo substituent is slightly superior to a chloro substituent, although this is not well documented. The products obtained by brominating the 3,4-dialkoxyl derivatives, which were found to have the 2-bromo-4,5-dialkoxyl configuration, were also found to be very active against *S. aureus* and *St. pyogenes*, but inferior with reference to *P. vulgaris*. The 2-bromo-3,4,5-trimethoxyl derivative is particularly active against *S. aureus*. Further bromination of this compound to give the 2,6-dibromo-3,4,5-trimethoxybenzylpyrimidine reduces the activity.

The antibacterial spectra of four of the most active of the 5-benzyl-2,4-diaminopyrimidines are shown in Table XI. In most instances the compounds are more active than sulfadiazine for a wide variety of pathogenic organisms. The use of one of these compounds (49–210) in combination with sulfadiazine, as well as in triple combination with sulfadiazine and other antimetabolites in *in vitro* tests, has been described.[3,4] All four of these compounds, as well as several others, have been subjected to extensive *in vivo* testing, and the results will be reported in future communications. Compound LXV, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (B.W. 56–72, trimethoprim), has been selected for detailed study and clinical trial. A preliminary report on its activity and mode of action as a metabolite antagonist has been presented.[5] In common with other 2,4-diaminopyrimidines, it is a competitor of folic and folinic acids in microorganisms which require these nutrilites and in *Streptococcus faecalis* can be shown to inhibit folic acid reductase.

Experimental[10]

Benzaldehydes.—Several new alkoxybenzaldehydes were prepared, using well known methods. These are characterized in Table I. Two of the procedures are described below.

3-Bromo-5-ethoxy-4-methoxybenzaldehyde (II).—3-Ethoxy-4-hydroxybenzaldehyde[11] was brominated according to the method of Dakin,[12] to produce the 5-bromo derivative. This was methylated in aqueous alkali with methyl sulfate, producing a crystalline product (II) which was recrystallized from ethanol.

3-Bromo-x-chloro-5-ethoxy-4-methoxybenzaldehyde (III).—Compound II,

(10) Melting points were taken in capillary tubes with a partial immersion thermometer and are not corrected. Ultraviolet absorption spectra were obtained on all final products; in all cases these support the benzylpyrimidine structures.

(11) Chemische Fabrik auf Aktien, Berlin, German Patent 81071 (1895). *Frdl.* **4**, 1281 (1894–7).

(12) H. D. Dakin, *Am. Chem. J.*, **42**, 477 (1909).

TABLE I

BENZALDEHYDES

Compound	Benzene substituent 3	4	5	x	Empirical formula	M.p. or b.p., °C. mm.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
I[a]	OC_2H_5	OC_3H_7-*n*			$C_{12}H_{16}O_3$	M.p. 41	69.20	7.75	69.59	7.74
II	OC_2H_5	OCH_3	Br		$C_{10}H_{11}BrO_3$	M.p. 53	46.35	4.28	45.96	4.46
III	OC_2H_5	OCH_3	Br	Cl	$C_{10}H_{10}BrClO_3$	M.p. 103	40.9	3.44	41.5	3.41
IV[b]	OCH_3	OC_3H_7-*n*	Br		$C_{11}H_{13}BrO_3$	B.p. 154 (1)	48.37	4.80	48.43	4.91
V[c]	OCH_3	OC_4H_9-*n*	Br		$C_{12}H_{15}BrO_3$	B.p. 141 (1)	50.19	5.27	50.61	5.87
VI[d]	OCH_3	OC_4H_9-*n*	OCH_3		$C_{13}H_{18}O_4$	M.p. 48.5–49.0	65.53	7.61	65.49	7.54

[a] From 3-ethoxy-4-hydroxybenzaldehyde[11] plus propyl iodide, according to the procedure of R. Dickinson, I. M. Heilbron, and F. Irving, *J. Chem. Soc.*, 1888 (1927). [b] From 5-bromovanillin,[12] plus propyl iodide, as in *a*. [c] From 5-bromovanillin,[12] plus butyl iodide, as in *a*. [d] From syringic aldehyde, plus butyl iodide, as in *a*.

TABLE II

CINNAMIC ACIDS

Compound	Benzene substituent 3	4	5	Empirical formula	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
VII[a]	OCH_3	OC_4H_9-*n*		$C_{14}H_{18}O_4$	154–155	67.18	7.25	67.26	7.4
VIII	OC_2H_5	OC_3H_7-*n*		$C_{14}H_{18}O_4$	152–153	67.18	7.25	67.5	7.2
IX	OCH_3	OC_3H_7-*n*	Br	$C_{13}H_{15}BrO_4$	108–109	49.54	4.72	49.41	5.03
X	OCH_3	OC_4H_9-*n*	Br	$C_{14}H_{17}BrO_4$	87	51.00	5.16	51.08	5.23

[a] From 1-*n*-butoxy-3-methoxybenzaldehyde; J. Böeseken and J. Creup, *Rec. trav. chim.*, **58**, 528 (1939).

(138 g., 0.56 mole) was mixed with 500 ml. of chloroform plus a small crystal of iodine. Chlorine gas was bubbled into the solution until the theoretical quantity (40 g.) had been added (1.5 hr.). After standing 3 days, the chloroform was evaporated and the product purified by extraction of soluble impurities with petroleum ether. The least soluble fraction (56 g., m.p. 103°) was found to be a monochloro derivative (III).

Cinnamic Acids.—These were prepared from the corresponding aldehydes by reaction with malonic acid in pyridine plus piperidine, according to the Doebner modification of the Perkin reaction.[6] The new derivatives which were obtained in analytically pure state are described in Table II. In many cases the products were obtained in sufficiently pure state to proceed directly with the next few steps without characterization at each point.

Cinnamic Esters.—New ethyl or methyl esters are characterized in Table III.

Hydrocinnamic Esters.—The cinnamic esters were reduced in ethanol with Raney nickel catalyst as described by Falco, *et al.*[1] New esters which were characterized are listed in Table IV.

Formylation of Hydrocinnamic Esters.—The procedure used followed that of Falco, *et al.*[1] The crude α-formyl derivatives were condensed directly with guanidine, without isolation of this intermediate.

2-Amino-5-benzyl-4-hydroxypyrimidines.—Again, the procedure of Falco, *et al.*,[1] was followed. New derivatives are found in Table V.

5-Benzyl-2,4-diaminopyrimidines.—Using the procedure of Falco, *et al.*,[1] for chlorination and amination, derivatives which are listed in Table VI were obtained. A number of the brominated benzylpyrimidines were obtained by bromination of the 5-benzyl-2,4-diaminopyrimidines, as described below. Nitro derivatives were obtained similarly.

2-Amino-4-chloro-5-(3,4,5-trimethoxybenzyl)pyrimidine (LXXXII).—2-Amino-4-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine was chlorinated by boiling with an excess of phosphoryl chloride,[1] and the product was isolated by the usual procedure of pouring on ice and neutralizing with ammonia. The precipitated product was then purified by recrystallization from 95% ethanol; m.p. 193°.

Anal. Calcd. for $C_{14}H_{16}ClN_3O_3$: C, 54.28; H, 5.21; N, 13.58. Found: C, 54.42; H, 5.28; N, 13.82.

5-(2-Bromo-4,5-dimethoxybenzyl)-2,4-diaminopyrimidine (LV).—This substance was prepared by two methods of synthesis: (a) from 2-bromo-4,5-dimethoxycinnamic acid[13] by complete synthesis (see Tables V and VI) and (b) from 2,4-diamino-5-(3,4-dimethoxybenzyl)pyrimidine.[1] Fifteen grams (0.576 mole) of the dimethoxybenzylpyrimidine was dissolved in 350 ml. of glacial acetic acid. A solution of 9.6 g. (0.06 mole) of bromine in 30 ml. of glacial acetic acid was added dropwise with stirring. The bromine color rapidly disappeared, and a white precipitate formed. After standing 3 hr., this was filtered off and washed with acetic acid and ether; weight, 22 g. This product, the *hydrobromide* of 5-(2-bromo-4,5-dimethoxybenzyl)-2,4-diaminopyrimidine, melted at 280–284° after recrystallization from ethanol. It was converted then to the free base by dissolving in hot water and precipitating with sodium hydroxide. After recrystallization from 50% ethanol with the aid of Darco G60, the white product melted at 260°. It showed no depression of melting point when mixed with the product of procedure (a).

Anal. Found: C, 46.08; H, 4.81; N, 16.80.

(13) F. B. Wittmer and L. C. Raiford, *J. Org. Chem.*, **10**, 527 (1945).

TABLE III

CINNAMIC ESTERS

Compound	Benzene substituent 3	Benzene substituent 4	Benzene substituent 5	Ester group	Empirical formula	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
XI	OCH_3	OC_4H_9-*n*		CH_3	$C_{15}H_{20}O_4$	103–104	68.16	7.63	68.37	7.22
XII[a]	OCH_3	$OCH_2C_6H_5$		CH_3	$C_{18}H_{18}O_4$	99–100	72.47	6.08	72.52	6.04
XIII	OC_2H_5	OC_3H_7-*n*		C_2H_5	$C_{16}H_{22}O_4$	88–89	69.04	7.97	68.7	7.9
XIV[b]	OC_2H_5	OCH_3	Br	C_2H_5	$C_{14}H_{17}BrO_4$	100–101	51.08	5.21	51.28	5.2
XV[b]	OCH_3	OC_4H_9-*n*	OCH_3	C_2H_5	$C_{17}H_{24}O_5$	B.p. 192–194 (1 mm.)	66.21	7.85	66.32	7.76

[a] From corresponding cinnamic acid: I. A. Pearl and D. L. Beyer, *J. Org. Chem.*, **16**, 216 (1951). [b] From aldehyde (Table I) *via* crude cinnamic acid.

TABLE IV

HYDROCINNAMIC ESTERS

Compound	Benzene substituent 3	Benzene substituent 4	Benzene substituent 5	Ester group	Empirical formula	M.p., °C. or b.p.°	mm.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
XVI	OCH_3	OC_4H_9-*n*		C_2H_5	$C_{16}H_{24}O_4$	173–174	(7)	68.54	8.63	68.58	8.07
XVII	OCH_3	$OCH_2C_6H_5$		CH_3	$C_{18}H_{20}O_4$	53–55		71.98	6.71	71.91	6.69
XVIII	OCH_3	OCH_3	Br	C_2H_5	$C_{13}H_{17}BrO_4$	170–173	(3)	49.22	5.40	49.58	5.32
XIX	OCH_3	OC_4H_9-*n*	OCH_3	C_2H_5	$C_{17}H_{26}O_5$	153	(0.5)	65.78	8.44	65.61	8.23

[a] From ester of corresponding cinnamic acid.[13]

TABLE V

2-AMINO-5-BENZYL-4-HYDROXYPYRIMIDINES

Compound	Benzene substituent 2	3	4	5	Empirical formula	M.p., °C.	Analyses, % Calcd. C	H	N	Found C	H	N
XX[a]			C_6H_5		$C_{17}H_{15}N_3O$	283–285	73.63	5.5	15.2	73.56	5.6	15.5
XXI[b]	OCH_3	OCH_3			$C_{13}H_{15}N_3O_3$	274–275	...	...	16.1	...	...	16.0
XXII[c]	OCH_3			Cl	$C_{12}H_{12}ClN_3O_2$	278–284	54.24	4.55	15.8	54.82	4.69	15.3
XXIII[d]		OCH_3	OC_3H_7-*i*		$C_{15}H_{19}N_3O_3$	238–239	62.27	6.62	14.5	62.19	6.83	14.5
XXIV		OCH_3	OC_4H_9-*n*		$C_{16}H_{21}N_3O_3$	210–211	63.35	6.98	13.8	63.49	6.94	13.7
XXV[e]		OCH_3	OC_5H_{11}-*i*		$C_{17}H_{23}N_3O_3$	234–235	64.35	7.30	..	64.45	7.11	..
XXVI		OCH_3	$OCH_2C_6H_5$		$C_{19}H_{19}N_3O_3$	204–205	..	..	12.5	..	..	12.6
XXVII[f]		Br	OCH_3		$C_{12}H_{12}BrN_3O_2$	245–248	..	..	13.6	..	..	13.3
XXVIII[g]		OC_2H_5	OC_2H_5		$C_{15}H_{19}N_3O_3$	215–216	62.27	6.62	14.5	62.43	6.55	14.4
XXIX[h]		Cl	Cl		$C_{11}H_9Cl_2N_3O$	264–266	48.91	3.36	15.6	49.27	3.23	15.1
XXX[i]		OCH_3		OCH_3	$C_{13}H_{15}N_3O_3$	241–244	..	..	16.1	..	..	16.1
XXXI[j]	Br		OCH_3	OCH_3	$C_{13}H_{14}BrN_3O_3$	238–240	45.88	4.14	12.4	45.97	4.14	12.2
XXXII[k]		OCH_3	OCH_3	OCH_3	$C_{14}H_{17}N_3O_4$	257–258	57.72	5.88	14.4	57.36	5.92	14.3
XXXIII		OCH_3	OC_4H_9-*n*	OCH_3	$C_{17}H_{23}N_3O_4$	255	61.24	6.95	12.6	61.63	6.99	12.5
XXXIV		OCH_3	OCH_3	Br	$C_{13}H_{14}BrN_3O_3$	239–241	..	..	12.4	..	..	12.0
XXXV[l]		OCH_3	OCH_3	Cl	$C_{13}H_{14}ClN_3O_3$	250–251	52.80	4.77	14.2	52.80	4.56	14.1
XXXVI[m]		OC_2H_5	OCH_3	Br	$C_{14}H_{16}BrN_3O_3$	252	47.41	4.55	11.9	47.69	4.55	11.9
XXXVII[n]		OCH_3	OC_3H_7-*n*	Br	$C_{15}H_{18}BrN_3O_3$	259–260	..	..	11.4	..	..	11.4
XXXVIII[n]		OCH_3	OC_4H_9-*n*	Br	$C_{16}H_{20}BrN_3O_3$	263–264	50.25	5.23	11.0	50.19	5.17	10.8

[a] From 4-phenylhydrocinnamic acid; R. M. Dodson and P. Sollman, *J. Am. Chem. Soc.*, **73**, 4197 (1951); ff. intermediates not purified. [b] From ethyl 2,3-dimethoxyhydrocinnamate; E. C. Horning, J. Koo, and G. N. Walker, *ibid.*, **73**, 5826 (1951). [c] From ethyl 5-chloro-2-methoxycinnamate; D. Chakravarti and B. Majumdar, *J. Indian Chem. Soc.*, **16**, 389 (1939); *C. A.*, **34**, 2348 (1940); ff. intermediates not purified. [d] From 3-methoxy-4-*i*-propoxybenzaldehyde, prepared according to R. Dickinson, *et al.* (see Table I, footnote *a*); ff. intermediates not purified. [e] From 4-*i*-amyloxy-3-methoxybenzaldehyde; G. Kubiczek, M. Pohl, and A. Smahel, *Monatsh. Chem.*, **77**, 52 (1947); ff. intermediates not purified. [f] From 3-bromo-4-methoxycinnamic acid; G. W. Gray, B.

Jones, and F. Marson, *J. Chem. Soc.*, 1417 (1956); ff. intermediates not purified. [g] From ethyl 3,4-diethoxyhydrocinnamate; K. Kindler and W. Peschke, *Arch. Pharm.*, **272**, 60 (1934). [h] From ethyl 3,4-dichlorocinnamate; Ng. Ph. Buu-Hoï; Ng. D. Xuong, Ng. H. Nam, F. Binon, and R. Royer, *J. Chem. Soc.*, 1358 (1953); ff. intermediates not purified. [i] From 3,5-dimethoxycinnamic acid; F. Manthner, *J. prakt. Chem.*, **110**, 125 (1925); ff. intermediates not purified. [j] From 2-bromo-4,5-dimethoxycinnamic acid[13]; ff. intermediates not purified. [k] From ethyl 3,4,5-trimethoxyhydrocinnamate; J. Koo, *J. Am. Chem. Soc.*, **75**, 1889 (1953). [l] From 5-chloro-3,4-dimethoxycinnamic acid[13]; ff. intermed. not purified. [m] See Table III; ff. intermed. not purified. [n] See Table II; ff. intermed. not purified.

TABLE VI

2,4-DIAMINO-5-BENZYLPYRIMIDINES

Compound	Benzene substituent 2	3	4	5	6	x	Empirical formula	M.p., °C.	Analyses, % Calcd. C	H	N	Found C	H	N
XXXIX[a]		OCH_3					$C_{12}H_{14}N_4O$	219–220	..	..	24.3	..	..	24.0
XL			C_6H_5				$C_{17}H_{16}N_4$	250–258	73.89	5.84	20.3	74.04	5.60	20.3
XLI	OCH_3	OCH_3					$C_{13}H_{16}N_4O_2$	193–197	59.98	6.20	21.5	60.0	6.16	21.2
XLII[b]	OCH_3		OCH_3				$C_{13}H_{16}N_4O_2$	171	59.98	6.20	21.5	59.84	6.03	21.6
XLIII	OCH_3			Cl			$C_{12}H_{13}ClN_4O$	169–171	54.44	4.95	21.5	54.60	4.99	21.5
XLIV[c]		OCH_3	OH				$C_{12}H_{14}N_4O_2 \cdot HCl \cdot {}^1/_2H_2O$	253–258	49.4	5.53	19.3	49.4	5.03	19.7
XLV[d]		OCH_3	OC_3H_7-*n*				$C_{15}H_{20}N_4O_2$	178–179	62.54	6.99	19.5	62.48	6.88	19.1
XLVI		OCH_3	OC_3H_7-*i*				$C_{15}H_{20}N_4O_2$	190–191	62.54	6.99	19.5	62.88	6.79	19.1
XLVII		OCH_3	OC_4H_9-*n*				$C_{16}H_{22}N_4O_2$	143–146	63.55	7.33	18.5	63.80	7.18	18.1
XLVIII		OCH_3	OC_6H_{11}-*i*				$C_{17}H_{24}N_4O_2$	146–147	..	..	17.7	..	..	17.8
XLIX		Br	OCH_3				$C_{12}H_{13}BrN_4O$	232–232.5	..	..	18.1	..	..	17.9
L[e]		OC_2H_5	OCH_3				$C_{24}H_{18}N_4O_2 \cdot H_2O$	197–198	57.52	6.90	19.2	58.06	6.79	19.3
LI		OC_2H_5	OC_2H_5				$C_{16}H_{20}N_4O_2 \cdot H_2O$	185–186	58.8	7.24	18.3	58.7	7.19	18.3
LII[f]		OC_2H_5	OC_3H_7-*n*				$C_{16}H_{22}N_4O_2$	160–162	..	..	18.5	..	..	18.9
LIII		Cl	Cl				$C_{11}H_{10}Cl_2N_4$	237–239	49.09	3.75	20.8	49.07	3.51	20.7
LIV		OCH_3		OCH_3			$C_{13}H_{16}N_4O_2 \cdot HCl$	273 dec.	52.51	5.77	..	52.12	6.05	..
LV[g]	Br		OCH_3	OCH_3			$C_{13}H_{15}BrN_4O_2$	260	46.03	4.46	16.5	46.12	4.67	16.3
LVI[h]	Br		OC_3H_7-*n*	OCH_3			$C_{15}H_{19}BrN_4O_2$	188	49.05	5.22	15.3	49.19	5.29	15.3
LVII[i]	Br		OC_3H_7-*i*	OCH_3			$C_{15}H_{19}BrN_4O_2$	193–194	49.05	5.22	15.3	49.39	5.17	15.2
LVIII[j]	Br		OC_4H_9-*n*	OCH_3			$C_{16}H_{21}BrN_4O_2$	181	50.40	5.55	14.7	50.40	5.74	14.5
LIX[k]	Br		OC_6H_{11}-*i*	OCH_3			$C_{17}H_{23}BrN_4O_2$	193	51.77	5.88	14.4	51.92	5.29	14.3
LX[l]	Br		OC_3H_7-*n*	OC_2H_5			$C_{16}H_{21}BrN_4O_2$	166–167	50.39	5.55	14.7	50.39	5.76	14.7
LXI[m]	OCH_3		OCH_3	Br			$C_{13}H_{15}BrN_4O_2$	242	46.01	4.45	16.5	46.39	4.35	16.6

LXII[n]	NO_2		OCH_3	OCH_3		$C_{13}H_{15}N_5O_4$	217	51.14	4.84	22.9	51.37	5.49	22.7
LXIII[o]		OCH_3	OC_3H_7-*n*		NO_2	$C_{15}H_{19}N_5O_4$	171–174	54.04	5.74	21.0	54.17	5.28	20.7
LXIV[p]		OCH_3	OC_4H_9-*n*		NO_2	$C_{16}H_{21}N_5O_4$	194–196	55.32	6.09	20.2	55.43	6.10	20.0
LXV		OCH_3	OCH_3	OCH_3		$C_{14}H_{18}N_4O_3$	199	57.92	6.25	19.3	57.95	6.38	19.4
LXVI		OCH_3	OC_4H_9-*n*	OCH_3		$C_{17}H_{24}N_4O_3$	163–164	61.42	7.28	16.9	61.93	7.29	17.1
LXVII		OCH_3	OCH_3	Br		$C_{13}H_{15}BrN_4O_2$	198–201	46.12	4.46	16.5	46.1	4.53	17.0
LXVIII		OCH_3	OCH_3	Cl		$C_{13}H_{15}ClN_4O_2$	188–189	52.97	5.01	19.0	53.06	5.21	18.9
LXIX		OC_2H_5	OCH_3	Br		$C_{14}H_{17}BrN_4O_2$	193–203	47.6	4.85	15.9	47.5	5.01	15.5
LXX		OCH_3	OC_3H_7-*n*	Br		$C_{15}H_{19}BrN_4O_2$	183	49.05	5.22	15.3	49.11	5.10	14.9
LXXI		OCH_3	OC_4H_9-*n*	Br		$C_{16}H_{21}BrN_4O_2$	178–179	50.50	5.55	14.7	50.57	5.50	14.4
LXXII[q]		OCH_3	$OCH_2C_6H_6$		Br	$C_{19}H_{19}BrN_4O_2$	200	54.94	4.61	13.5	54.96	4.50	13.4
LXXIII[r]	Br	OCH_3	OCH_3	OCH_3		$C_{14}H_{17}BrN_4O_3$	192–193	45.53	4.64	15.2	45.90	4.67	14.8
LXXIV[s]	Br	OCH_3	OC_4H_9-*n*	OCH_3		$C_{17}H_{23}BrN_4O_3 \cdot HBr$	265 dec.	41.47	4.91	11.4	41.72	4.72	11.7
LXXV[t]		OCH_3	OCH_3	Br	Br	$C_{13}H_{14}Br_2N_4O_2$	225–227	37.34	3.38	13.4	37.65	3.24	13.4
LXXVI[u]		OC_2H_5	OCH_3	Br	Cl	$C_{14}H_{16}BrClN_4O_2 \cdot HCl$	240 dec.	..	..	13.2	..	..	12.9
LXXVII[v]	Br	OCH_3	OCH_3	OCH_3	Br	$C_{14}H_{16}Br_2N_4O_3$	225	37.52	3.60	12.5	37.74	3.34	12.6

[a] From ethyl 3-methoxyhydrocinnamate; A. Cohen, *J. Chem. Soc.*, 429 (1935); ff. intermed. not purified. [b] From ethyl 2,4-dimethoxycinnamate; W. H. Perkin and E. Schiess, *ibid.*, **85**, 159 (1904); ff. intermed. not purified. [c] From crude 4-benzyloxy-3-methoxy derivative by reduction with palladium on charcoal. [d] From 3-methoxy-4-*n*-propoxybenzaldehyde (see footnote *g*, Table V); ff. intermed. not purified. [e] From 3-ethoxy-4-methoxycinnamic acid; H. Shishido, *Bull. Chem. Soc. Japan*, **12**, 419 (1937); *C.A.* **32**, 944 (1938); ff. intermed. not purified. [f] See Table III; ff. intermed. not purified. [g] By two methods of synthesis; see experimental section. [h] By bromination of XLV, as described for LV. Calcd. Br, 21.76; found, 21.75. [i] By bromination of XLVI, as described for LV. [j] By bromination of XLVII, as described for LV. Calcd. Br, 20.96; found, 21.13. [k] By bromination of XLVIII, as described for LV. [l] By bromination of LII, as described for LV. [m] By bromination of XLII, as described for LV. [n] By nitration of 3,4-dimethoxy derivative; see experimental section. [o] By nitration of XLVI. [p] By nitration of XLVII. [q] By bromination of crude 3-methoxy-4-benzyloxy derivative, as described for LV. [r] By bromination of LXV; see experimental section. [s] By bromination of LXVI, as described for LXXIII. [t] By bromination of LXVII, as described for LV. [u] From 3-bromo-x-chloro-5-ethoxy-4-methoxybenzaldehyde; see exptl. section; ff. intermed. not purified. [v] By bromination of LXXIII; see exptl.

TABLE VII

2-AMINO-4-SUBSTITUTED-AMINO-5-BENZYLPYRIMIDINES

Compound	Pyrimidine substituents 2	4	Benzene substituents 3	4	5	Empirical formula	M.p., °C.	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
LXXVIII	NH_2	$NHCH_3$	OCH_3	OCH_3		$C_{14}H_{18}N_4O_2$	218–221	..	..	20.4	..	..	20.2
LXXIX	NH_2	$NHCH_3$	OCH_3	OCH_3	Br	$C_{14}H_{17}BrN_4O_2 \cdot HCl$	185–187	..	..	14.4	..	..	14.0
LXXX	NH_2	$NHCH_3$	OCH_3	OCH_3	OCH_3	$C_{15}H_{20}N_4O_3$	168–170	59.19	6.62	18.4	59.13	6.62	18.3
LXXXI	NH_2	$NH(CH_2)_3N(CH_3)_2$	OCH_3	OCH_3	OCH_3	$C_{19}H_{29}N_5O_3$	127–128	60.77	7.78	18.6	60.69	7.79	18.3

This product and that obtained by complete synthesis were converted to *picrate* salts. Both melted at 243° and showed no depression in melting points on admixture. Chromatograms were run on both free bases in isopropyl alcohol: 5% ammonium sulfate in water (5:95). Only one spot was obtained in each case, with identical R_f values of 0.55. Both had ultraviolet absorption maxima in 0.1 *N* HCl at 277 mμ (ϵ_m = 7960), and in pH 11 Sørensen glycine–NaOH buffer at 231 mμ (ϵ_m = 23,750) and 286 mμ (ϵ_m = 11,200). The only product thus isolated by direct bromination was the 2-bromo-4,5-dimethoxyl derivative. The corresponding 3-bromo-4,5-dimethoxyl derivative (LXVII) was converted to its hydrobromide. This melted at 263–264°, and depressed the melting point in admixture with the above described hydrobromide of the 2-bromo-4,5-dimethoxyl derivative to 253–254°.

Several other 3,4-dialkoxybenzylpyrimidines were brominated in a manner similar to LV, as shown in Table VI. Single products were obtained in all cases. The structure of these products was not proven; by analogy, the 2-bromo-4,5-dialkoxyl configuration is assumed as being most reasonable. In brominating the 2,4-dimethoxyl derivative, it is assumed that the product was the 5-bromo derivative (LXI); proof was not obtained.

5-(2-Bromo-3,4,5-trimethoxybenzyl)-2,4-diaminopyrimidine (LXXIII).—One gram (0.00345 mole) of 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (LXV) was dissolved in 15 ml. of glacial acetic acid. To this was added dropwise a solution of 0.56 g. (0.0035 mole) of bromine in 10 ml. of glacial acetic acid. Rapid decolorization occurred, and a white precipitate formed, which became very heavy as the addition neared completion. The product was isolated and slurried in water, in which it was insoluble. Upon addition of sodium hydroxide a gum formed which soon solidified. This was recrystallized from dilute ethanol yielding LXXIII as white crystals.

2,4-Diamino-5-(2,6-dibromo-3,4,5-trimethoxybenzyl)pyrimidine (LXXVII).—One gram (0.00278 mole) of 5-(2-bromo-3,4,5-trimethoxybenzyl)-2,4-diaminopyrimidine (LXXIII) was dissolved in 15 ml. of glacial acetic acid and brominated as above for LXXIII. The solution turned yellow and only a trace of precipitate formed. After cooling for several days, a yellow precipitate was present. This was isolated and slurried in water; on adding alkali, the yellow color slowly disappeared, yielding an off-white precipitate. This was recrystallized from 90% ethanol plus a trace of ammonia, yielding LXXVII as white crystals.

When 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine was brominated in considerably more dilute solution (1 g./100 ml. acetic acid) with one mole of bromine, the main product isolated was the dibromo rather than the monobromo derivative.

Nitration of 2,4-Diamino-5-(3,4-dimethoxybenzyl)pyrimidine.—Thirteen grams (0.05 mole) of 2,4-diamino-5-(3,4-dimethoxybenzyl)pyrimidine was dissolved in 200 ml. of glacial acetic acid and cooled to 18°. A cooled solution of 6.4 ml. (0.10 mole) of concentrated nitric acid (70%) in 40 ml. acetic acid was prepared. One half of this was mixed with the well-stirred pyrimidine solution and the other half then was poured in more slowly, over a 2 min. period. The solution turned yellow, and a white precipitate began to form before all the nitric acid had been added. This precipitate gradually changed color, becoming tan and quite thick. The mixture was allowed to stir at room temperature for 3 hr., and then was filtered and washed with acetic acid and ether; dry weight, 16 g. This was then slurried in water plus 10 ml. of 5 *N* NaOH, and warmed to 50° for 30 min. Some of the

substance dissolved, yielding a reddish-brown solution. The mixture was cooled, filtered, and the yellow insoluble fraction (A) washed well with water; dry weight 12.5 g. Neutralization of the alkaline filtrate yielded a very small gelatinous precipitate (B), which was not investigated further. The yellow product (A) was very insoluble in hot ethanol and hot water, but was soluble to the extent of 1 g./24 ml. in boiling 80% ethanol. It was likewise very insoluble in hot acetone, but easily soluble in hot aqueous acetone solution. After two recrystallizations from dilute ethanol with the aid of charcoal, followed by rapid cooling and filtration, bright shiny yellow crystals were obtained (9.6 g.); m.p. 217° (LXII). The substance was unstable to light; rapid darkening and reddening occurred, and the melting point was lowered to 209–211° after standing in daylight several days.

When one mole of nitric acid was employed in this preparation, the colorless nitrate of the pyrimidine was precipitated, and no nitration of the benzene ring took place. Similarly, when two moles of nitric acid were employed, but added very slowly, the colorless nitrate precipitated first, giving a two phase system which reacted further with difficulty. Under the conditions used here, some nitrate precipitated first, but its formation was minimized by the rapid addition. When the reaction mixture was warmed to 45° slightly lower yields were obtained. In all cases, some starting material was isolated along with the product. This was more soluble in alcoholic solutions than the nitro derivative, and remained in the mother liquors, particularly when the recrystallization media were cooled rapidly. When sulfuric rather than acetic acid was tried as the reaction solvent, sulfonation occurred.

By analogy to the bromination reactions, this product was assigned the 4,5-dimethoxy-2-nitro structure. Rigorous proof was not obtained. Nitration of the 3,4,5-trimethoxy derivative under similar conditions led to extensive decomposition. No nitro derivative was isolated. Nitration of the 3-methoxy-4-propoxy and 3-methoxy-4-butoxybenzylpyrimidines gave traces of secondary products which possibly were isomeric nitro derivatives. These were not investigated further. No assignment of position is made to these nitro compounds (LXIII, LXIV).

5-(3,4-Dimethoxybenzyl)-4-hydroxy-2-mercaptopyrimidine (LXXXIII).—Fifty grams (0.21 mole) of ethyl 3,4-dimethoxyhydrocinnamate was formylated using ethyl formate plus sodium (4.8 g., 0.21 mole) in ether as described by Falco, *et al.*[1] This crude product was treated with 15.8 g. (0.21 mole) of thiourea in 200 ml. of absolute ethanol following the technique of Johnson and Ambelang.[8] There was obtained 10.7 g. (18%) of LXXXIII, which after recrystallization from 50% ethanol melted at 229–231°.

Anal. Calcd. for $C_{13}H_{14}N_2O_3S$: N, 10.07. Found: N, 9.85.

5-(3,4-Dimethoxybenzyl)-4-hydroxy-2-methylthiopyrimidine (LXXXIV).—This product was obtained by methylation of LXXXIII with methyl sulfate in aqueous alkali; needles from 50% methanol, m.p. 197–205°.

Anal. Calcd. for $C_{14}H_{16}N_2O_3S$: C, 57.51; H, 5.52; N, 9.58. Found: C, 57.79; H, 5.94; N, 9.40.

4-Amino-5-(3,4-dimethoxybenzyl)-2-hydroxypyrimidine (LXXXV).—5-(3,4-Dimethoxybenzyl)-4-hydroxy-2-methylthiopyrimidine (LXXXIV) (7.5 g.) was converted to the crude 4-chloro derivative with phosphoryl chloride in the usual manner, followed by treatment with a saturated solution of alcoholic ammonia at 100° in an autoclave for 16 hr. The resultant crude 4-amino-2-methylthio derivative (4.4 g.) was heated in an open dish with 75 ml. of concd. hydrochloric

acid on the steam bath until the solvent had all evaporated, yielding 4-amino-5-(3,4-dimethoxybenzyl)-2-hydroxypyrimidine; this melted at 285° dec. after recrystallization from dilute ethanol.

Anal. Calcd. for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.1. Found: C, 59.85; H, 5.86; N, 15.6.

2,4-Dihydroxy-5-(4-methoxybenzyl)pyrimidine (LXXXVI).—4-Hydroxy-2-mercapto-5-(4-methoxybenzyl)pyrimidine[9] was treated with chloroacetic acid by the method of Wheeler and Liddle[14] to replace the 2-mercapto with a 2-hydroxy group. The product was recrystallized from 50% ethanol, and then melted at 285° dec.

Anal. Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21. Found: C, 62.00; H, 5.00.

2-Amino-5-(4-chlorobenzyl)-4-mercaptopyrimidine (LXXXVII).—Fifteen grams of 2-amino-5-(4-chlorobenzyl)-4-hydroxypyrimidine[1] was stirred for 2 hr. at 155–170° with 45 g. of phosphorus pentasulfide in 100 ml. of tetralin. After cooling, the product was filtered off, and washed with petroleum ether, then boiled in water to destroy excess P_2S_5. After purification by reprecipitation from ammoniacal solution, and two recrystallizations from ethanol, there was recovered 4.2 g. of yellow crystals melting at 248° dec.

Anal. Calcd. for $C_{11}H_{10}ClN_3S$: C, 52.48; H, 4.00; N, 16.7. Found: C, 52.62; H, 4.11; N, 16.5.

4-Alkylamino-2-amino-5-benzylpyrimidines.—These derivatives were prepared from the corresponding 4-chloro derivatives by treatment with alkylamines. In the case of the volatile amines, the compound was heated at 120° for 6 hr. in an alcoholic solution which was saturated with the amine. The solvent was then distilled off, and the product was recrystallized from dilute ethanol. In the case of a higher boiling amine, the compound was refluxed with an excess of the amine for 3 hr., and the product was isolated by pouring the mixture into water, and recrystallizing from dilute ethanol as above. The products are characterized in Table VII.

Ultraviolet Absorption Spectra.—Representative ultraviolet absorption spectra are shown in Table VIII. Absorptions were measured at a concentration of 10 mg./l. on the Beckman Model DU spectrophotometer in 0.1 *N* hydrochloric acid and Sørensen glycine-sodium hydroxide buffer at pH 11.0.

In Vitro **Antibacterial Screening Methods and Materials. (a) M.I.C. Test** (see Tables IX–XI).—Compounds were converted to the soluble isethionates by suspending 20 mg. of the base in 5 ml. of water and adding the minimum quantity of normal isethionic acid to give a clear solution. One compound (XL) did not form a soluble isethionate, and was therefore used as a suspension. The solutions were sterilized by heating at 60° for 1 hr. and then diluted two-fold in the test medium. In the assays in which the test medium was nutrient agar, the dilutions were made in 10-ml. quantities, and subdivided into series containing 0.5-ml. quantities, one series being used for each of the test organisms. To each tube was added an equal volume of the nutrient broth containing 2.5% agar, and after the medium had set in a sloped position, the tubes were inoculated with the test organism by running over the surface of the medium 0.05 ml. of 10^{-2} or 10^{-6} dilution of a 24 hr. nutrient broth culture of the organism. The smaller inoculum was used for the members of the *Enterobacteriaceae* family, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The dilutions were made in 3–5 day-old oxalated horse blood for the tests with *Streptococcus pyogenes* and in saline, to which was

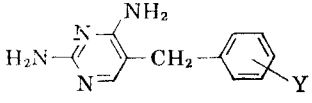
(14) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

TABLE VIII: ULTRAVIOLET ABSORPTION SPECTRA OF 5-BENZYLPYRIMIDINES: Sh = Shoulder

Compound	Pyrimidine substituents		Benzene substituents					pH 1				pH 11			
								Maximum		Minimum		Maximum		Minimum	
								λ, mμ	E × 10^{-3}	λ, mμ	E × 10^{-3}	λ, mμ	ε × 10^{-3}	λ, mμ	ε × 10^{-3}
	2	4	2	3	4	5	6								
LXV	NH_2	NH_2		OCH_3	OCH_3	OCH_3		271	6.25	258	5.05	230	19.2	258	2.64
												287	7.85		
LXXIII	NH_2	NH_2	Br	OCH_3	OCH_3	OCH_3		273	7.25	260	6.1	233	21.2	260	3.25
												286	8.7		
LXXVII	NH_2	NH_2	Br	OCH_3	OCH_3	OCH_3	Br	273	5.15	260	4.8	232	29.0	263	4.48
												287	8.7		
LXXX	NH_2	$NHCH_3$		OCH_3	OCH_3	OCH_3		271	7.6	260	7.1	231	16.0	263	3.6
												287.5	7.5		
LXXXI	NH_2	$NH(CH_2)_3N(CH_3)_2$		OCH_3	OCH_3	OCH_3		270	8.7	260	7.9	232	16.4	262	3.8
												289	8.8		
LXXXII	NH_2	Cl		OCH_3	OCH_3	OCH_3		310	4.33	284	2.9	234	24.2	260	2.2
												299	3.4		
XXXII	NH_2	OH		OCH_3	OCH_3	OCH_3		262	9.2	252	8.6	229	16.3	257	5.3
												289	7.1		
LXXXIII	SH	OH		OCH_3	OCH_3			280	20.4	247	6.7	232	15.9	248	11.8
												263	15.1	292.5	7.5
												308	9.7		
LXXXIV	SCH_3	OH		OCH_3	OCH_3			253	10.0	245	9.7	Sh245	11.5	265	7.2
										259	9.8	282	11.2		
LXXXV	OH	NH_2		OCH_3	OCH_3			282.5	7.3	249	1.0	229	11.7	255	3.0
												277	5.8		
LXXXVI	OH	OH			OCH_3			267	8.3	244	5.5	222	14.8	247	3.3
												271	6.2		
LXXXVII	NH_2	SH			Cl			Sh251	8.2	291	3.8	Sh261	7.0	287	2.8
								330	13.1			317	11.0		

TABLE IX

ANTIBACTERIAL ACTIVITY *in vitro* OF 5-BENZYL-2,4-DIAMINO-6-UNSUBSTITUTED-PYRIMIDINES[a]



Compound no. or ref.	Benzene substituents (Y)	Minimum inhibitory concentration, μg./ml., in: Nutrient agar *P. vulgaris*	Nutrient agar *S. aureus*	Nutrient agar *St. pyogenes*	Whole blood *St. pyogenes*
LXV	3,4,5-Trimethoxy	1	0.5	0.25	0.5
[b]	3,4-Dimethoxy	4	1	2	4
XLIX	3-Bromo-4-methoxy	8	1	1	2
XLIV	3-Methoxy-4-hydroxy	8	<2	<2	<1
XLV	3-Methoxy-4-*n*-propoxy	10	0.25	0.5	2.5
L	3-Ethoxy-4-methoxy	12	0.6	1	2.5
LXII	2-Nitro-4,5-dimethoxy	12	0.3	4	6
LXVII	3,4-Dimethoxy-5-bromo	16	1	1	2
LIV	3,5-Dimethoxy	16	2	4	16
XLVI	3-Methoxy-4-*i*-propoxy	16	1	0.5	1
LI	3,4-Diethoxy	20	<1	<1	<1
XXXIX	3-Methoxy	25	6	2	12
LXIX	3-Ethoxy-4-methoxy-5-bromo	25	0.25	0.5	0.75
LXVIII	3,4-Dimethoxy-5-chloro	32	0.5	0.25	2
XLIII	2-Methoxy-5-chloro	32	1.5	0.5	1.5
LXXIII	2-Bromo-3,4,5-trimethoxy	32	0.06	1	8

XLVII	3-Methoxy-4-*n*-butoxy	32	0.5	0.25	2
[b]	4-Chloro	50	12	4	12
[b]	4-Methyl	50	12	2	3
[b]	4-Dimethylamino	50	25	4	6
[b]	-(Unsubstituted)	50	25	4	12
LII	3-Ethoxy-4-*n*-propoxy	100	0.5	0.5	1
LIII	3,4-Dichloro	100	2	0.5	4
LXXVI	3-Ethoxy-4-methoxy-5-bromo-x-chloro	125	0.25	0.5	2
LXVI	3,5-Dimethoxy-4-*n*-butoxy	125	1	0.25	2
[c]	3-Methoxy-4-benzyloxy	125	0.5	0.5	3
[d]	3,4-Methylenedioxy	125	16	0.5	16
LVII	2-Bromo-4-*n*-propoxy-5-methoxy	250	0.12	0.5	8
XII	2,3-Dimethoxy	250	16	<2	<1
[b]	4-Methoxy	320	20	<2	8
LXIV	3-Methoxy-4-*n*-butoxy-x-nitro	500	0.12	4	62
XLVIII	3-Methoxy-4-*i*-amyloxy	500	0.5	0.25	8
LXX	3-Methoxy-4-*n*-propoxy-5-bromo	500	0.5	0.25	4
LXIII	3-Methoxy-4-*n*-propoxy-x-nitro	500	0.5	16	62
LV	2-Bromo-4,5-dimethoxy	500	1	1	4
LVIII	2-Bromo-4-*n*-butoxy-5-methoxy	1000	0.12	0.25	4
XL	4-Phenyl	1000	5	1	2.5
LXXI	3-Methoxy-4-*n*-butoxy-5-bromo	>1000	2	1	16
	Sulfadiazine standard varied as ff:	2–4	4–8	32–125	32–125

[a] Listed in decreasing order of activity *vs. P. vulgaris*. [b] See Ref. 1. [c] Crude intermediate for XLIV. [d] P. S. Stenbuck, R. Baltzly, and H. M. Hood, Abs., 138th Meeting, American Chemical Society, Sept. 1960, p. 18. To be published.

TABLE X

ANTIBACTERIAL ACTIVITY *in vitro* OF MISCELLANEOUS 5-BENZYLPYRIMIDINES

Ref.	Pyrimidine substituents 2	4	6	Benzene substituents 2'	3'	4'	5'	x	Minimum inhibitory concentration, μg./ml., in: Nutrient agar — *P. vulgaris*	*S. aureus*	Whole blood — *St. pyogenes*	*St. pyogenes*
[a]	NH_2	NH_2	CH_3			NH_2			1000	62.0	8.0	31.0
[a]	NH_2	NH_2	CH_3			OCH_3			500	31.0	4.0	4.0
[a]	NH_2	NH_2	CH_3		—OCH_2O—				500	31.0	2.0	16.0
[a]	NH_2	NH_2	CH_3		CH_3				500	31.0	4.0	16.0
[a]	NH_2	NH_2	CH_3			CH_3		Br	>1000	30.0	4.0	4.0
[a]	NH_2	NH_2	CH_3			Cl			250	16.0	4.0	4.0
[a]	NH_2	NH_2	CH_3	Cl		Cl			125	4.0	4.0	8.0
[a]	NH_2	OH	..		OCH_3	OCH_3			500	500	125	1000
LXXXII	NH_2	Cl	..		OCH_3	OCH_3	OCH_3		>250	>250	>250	..
[b]	NH_2	NH_2	OH		OCH_3	OCH_3			1000	>1000	500	>1000
LXXIX	NH_2	$NHCH_3$	..		OCH_3	OCH_3	Br		>1000	32.0	16	500
LXXVII	NH_2	$NHCH_3$	..		OCH_3	OCH_3			>1000	62.0	1000	>1000
LXXXV	OH	NH_2	..		OCH_3	OCH_3			1000	>1000	>1000	>1000
LXXX	NH_2	$NHCH_3$	..		OCH_3	OCH_3	OCH_3		1000	1000	250	>1000

[a] See Ref. 1. [b] Prepared by Dr. Norman Whittaker, Wellcome Research Laboratories, Beckenham, England.

TABLE XI

ANTIBACTERIAL SPECTRUM *in vitro* OF 2,4-DIAMINO-5-BENZYLPYRIMIDINES

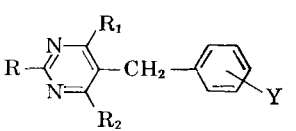
Organism	Medium	Minimum Inhibitory Concentration, μg./ml. B.W. 56-72[a]	B.W. 49-210[b]	B.W. 53-16[c]	B.W. 51-90[d]	Sulfadiazine
St. pyogenes	Nutrient agar	0.25	2.0	1.0	1.0	250
St. pyogenes	Whole blood	0.5	4.0	2.0	2.0	125
S. aureus	Nutrient agar	0.5	1.0	1.0	1.0	2.0
Sal. typhosa	Nutrient agar	0.5	0.12	1.0	1.0	2.0

E. coli	Nutrient agar	1.0	0.25	4.0	4.0	4.0
Vibrio comma	Nutrient agar	1.0	0.25	1.0	2.0	1000
Shig. dysenteriae	Nutrient agar	0.5	0.25	2.0	2.0	2.0
Ps. aeruginosa	Nutrient agar	125	125	500	500	31
P. vulgaris	Nutrient agar	1.0	4.0	8.0	16.0	4.0
St. agalactiae	Nutrient agar	4.0	4.0	8.0	8.0	>1000
Ery. rhusiopathiae	Nutrient agar + 10% serum	16.0	62.0	31.0	125	>1000
Past. boviseptica	Nutrient agar	0.5	0.06	0.25	0.5	8.0
Cl. perfringens	Nutrient agar	62.0	250	125	16.0	4.0
C. pyogenes	Nutrient agar + 10% serum	1.0	4.0	8.0	2.0	>1000
Mon. albicans	Nutrient agar	>1000	>1000	>1000	>1000	Undecylenic acid 250
M. tuberculosis (var. hominis)	Peizer and Schecter	250	500	6.0	>1000	Isoniazid 0.06

[a] LXV, 3,4,5-trimethoxybenzyl. [b] 3,4-Dimethoxybenzyl (see Ref. 1). [c] XLIX, 3-bromo-4-methoxybenzyl. [d] LXVII, 3,4-dimethoxy-5-bromobenzyl.

TABLE XII

ANTIBACTERIAL ACTIVITY *in vitro* OF 5-BENZYLPYRIMIDINES



Compound no. or ref.	Benzene substituents (Y)	R	R_1	R_2	Concn., mg./ml.	Zones of inhibition, mm. *P. vulgaris*	*S. aureus* 203	*E. coli*
LIX	2-Bromo-4-*i*-amyloxy-5-methoxy	NH_2	NH_2	H	1	28[e]	26	29
LXV	3,4,5-Trimethoxy	NH_2	NH_2	H	1	23	23	30
					0.1	0	13	26
"	3,4-Dimethoxy	NH_2	NH_2	H	1	23	20	29
					0.1	0	12	24

LXI	2,4-Dimethoxy-5-bromo	NH_2	NH_2	H	1	tr.	31	27
LVII	2-Bromo-4-*i*-propoxy-5-methoxy	NH_2	NH_2	H	1	0	30	26
LX	2-Bromo-4-*n*-propoxy-5-ethoxy	NH_2	NH_2	H	1	tr.	30	25
LXXII	3-Methoxy-4-benzyloxy-x-bromo	NH_2	NH_2	H	1	tr.	27	24[p]
[b]	3,4-Methylenedioxy	NH_2	NH_2	H	1	15[p]	18	23
LXXIV	2-Bromo-3,5-dimethoxy-4-*n*-butoxy	NH_2	NH_2	H	1	0	27	18[p]
[b]	3-Methoxy-4-(1-methylpropoxy)	NH_2	NH_2	H	1	tr.	19	26[p]
LXXVII	2,6-Dibromo-3,4,5-trimethoxy	NH_2	NH_2	H	1	tr.	19[p]	25[p]
[c]	2-Bromo-4,5-methylenedioxy	NH_2	NH_2	H	1	14[p]	17[p]	21[p]
[b]	3-Ethoxy	NH_2	NH_2	H	1	0	15.5	25
XLII	2,4-Dimethoxy	NH_2	NH_2	H	1	0	15[p]	19[p]
[c]	4-Fluoro	NH_2	NH_2	H	1	0	0	21[p]
LXXXVII	4-Chloro	NH_2	SH	H	1	tr.	14	14
LXXXI	3,4,5-Trimethoxy	NH_2	$NH(CH_2)_3N(CH_3)_2$	H	1	12	0	12
LXXXVI	4-Methoxy	OH	OH	H	1	12	12	13
[c]	2,4,6-Trimethyl	NH_2	NH_2	H	1	0	0	17[a]
[b]	3-Methoxy-4-octyloxy	NH_2	NH_2	H	1	0	14	..
[a]	2-Chloro	NH_2	NH_2	CH_3	2	..	12[a]	0
[a]	4-Chloro	NH_2	NH_2	CH_3	1	0	0	0
LXXX	3,4,5-Trimethoxy	NH_2	$NHCH_3$	H	1	0	0	0
LXXXIII	3,4-Dimethoxy	SH	OH	H	1	12[p]	12	0
XXXII	3,4,5-Trimethoxy	NH_2	OH	H	1	12[p]	0	0
[d]	3,4-Dimethoxy	SH	NH_2	H	1	0	0	0

[a] See Ref. 1. [b] See Table IX, footnote *d*. [c] Prepared by Dr. Fred Gerns, Wellcome Research Laboratories, Tuckahoe, N. Y. [d] Prepared by Dr. Eugene Grivsky, Wellcome Research Laboratories, Tuckahoe, N. Y. Syntheses of *c* and *d* to be published.

added 9 times the volume of the molten Peizer and Schecter medium, for the tests with *Mycobacterium tuberculosis*. The starting dilution of the drug was either 1000 μg./ml. or 100 μg./ml., depending on the activity shown in preliminary tests.

The nutrient agar was prepared from horse muscle, a papain digest of the muscle being added to a watery infusion from 300 g. of muscle per liter to give a total nitrogen content of 1.5 g./l., and the pH value adjusted to 7.6; the assays with *Corynebacterium pyogenes* and *Erysipelothrix rhusiopathiae* were made in the nutrient agar supplemented with 10% horse serum, and those with *Mycobacterium tuberculosis* were made in Peizer and Schecter egg-agar medium.[15]

(b) **Agar Plating Test** (see Table XII).—Compounds were dissolved in aqueous medium by adding a minimum amount of hydrochloric or lactic acid. The concentration of compound employed was 1 mg./ml., except where otherwise noted. Discs of Whatman #1 filter paper, 10 mm. in diameter, were dipped into the test solutions. It was found that approximately 0.02 ml. of aqueous solution was thus absorbed, or 20 γ of test compound, at the above concentration. The discs were then placed on agar plates containing P.C. (Phenol Coefficient) medium.[16] This consisted of a base layer and a seed layer which had previously been inoculated with the bacteria in question.

After incubation at 37° for 48 hr., zones of inhibition were measured. These are expressed as the outside total diameter, in mm., of the clear zone of inhibition surrounding the paper disc. Thus, a zone which extends 1 mm. on each side beyond the paper disc would be said to have a zone measuring 12 mm. Where no inhibition was observed beyond the edges of the disc, the zone is expressed as zero. Where inhibition was not complete throughout the zone, the diameters are expressed with a superscript "p" to indicate partial inhibition.

Acknowledgments.—The authors are indebted to Samuel W. Blackman and to Veronica Purdey for the microanalyses reported here, to Elizabeth Pappas Katsikis, Eva Hart Gold, and Lorraine Schloemer for technical assistance, to Nicholas Rigopolous, and Anthony Iannotti for the antibacterial assays, and to our colleagues Margaret Barnett, Samuel Singer and Marion B. Sherwood, for many helpful suggestions.

(15) L. R. Peizer and C. Schechter, *Am. J. Clin. Pathol.*, **20**, 682 (1950).

(16) This medium was prepared as follows: to a mixture of 10 g. of Armour Peptonium Siccum, 5 g. of sodium chloride, and 3 g. of beef extract (Difco) was added glass-distilled water to 1 l. The pH was adjusted to 7.3 with 2 *N* sodium hydroxide, 1.5% agar agar added, and the mixture autoclaved at 1.055 kg./cm.^{2} pressure for 15 min.