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Pyrimidines. XI. Structural Variations of 2,4-Diamino-6-(halogenoanilino)-5-nitrosopyrimidines[1]

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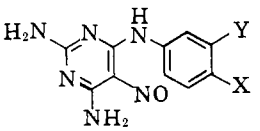
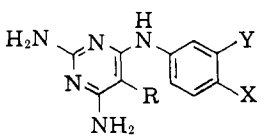
Derivatives of 2,4-diamino-6-(halogenoanilino)-5-nitrosopyrimidines (I) have been synthesized. These include compounds in which the 5-nitroso group of I has been replaced by other moieties (ethyl, nitro, bromo, cyano, carbamoyl, and ring nitrogen) as well as those in which the hydrogen atoms of the primary and secondary amino functions of I have been substituted by alkyl groups. Both areas furnish compounds that possess activity in the Adenocarcinoma-755 and Sarcoma-180 tumor systems.

The carcinostatic activity of 2,4-diamino-6-(*p*-bromoanilino)-5-nitrosopyrimidine (I, X = Br, Y = H), 2,4-diamino-6-(3,4-dichloroanilino)-5-nitrosopyrimidine (I, X, Y = Cl), and 2,4-diamino-6-(*p*-iodoanilino)-5-nitrosopyrimidine (I, X = I, Y = H) in the adenocarcinoma-755 tumor system[2] has led to a more thorough and systematic study of the synthesis of related compounds.

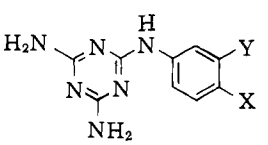


I

The study was divided into two general areas. The first involves the replacement of the 5-nitroso group by ethyl, nitro, bromo, cyano, and carbamoyl groups (illustrated as II) which possess size or electron-withdrawing effects similar to the nitroso group. In addition, some 2,4-diamino-6-(halogenoanilino)-*s*-triazines (III) are also included in this area of study since the



II



III

additional nitrogen causes the same electronic effect as that of a nitroso group at the same position. The 5-ethyl derivatives (II, R = C_2H_5) were prepared by the fusion of the corresponding aniline and 2,4-diamino-6-chloro-5-ethylpyrimidine.[3] The substituted *s*-triazines (III) were obtained from 2-chloro-4,6-diamino-*s*-triazine[4] by the method of Banks.[5]

2,4-Diamino-6-chloropyrimidine[6] was readily nitrated in a mixture of red fuming nitric acid and sulfuric acid at 20°, a procedure similar to that described for the preparation of 4-chloro-5-nitro-2,6-pyrimidinediol.[7] The resulting 2,4-diamino-6-chloro-5-nitropyrimidine was then treated with the appropriate anilines to yield the corresponding 5-nitropyrimidines (II, R = NO_2).

Bromination of 2,4-diamino-6-chloropyrimidine with bromine and acetic acid in the presence of sodium acetate furnished a good yield of 2,4-diamino-5-bromo-6-chloropyrimidine (IVa). Subsequent reaction of IVa and a substituted aniline afforded the desired 5-bromo analog (II, R = Br).

The 5-cyano derivatives (II, R = CN) were prepared from 2,4,6-trichloro-5-pyrimidinecarbonitrile[8] (IVb) which was treated with an aniline at 0–5° to give a monoanilino-substituted pyrimidine.[9] The structure for the latter compound was designated as 2,4-dichloro-6-(substituted-anilino)-5-pyrimidinecarbonitrile since the order of reactivity of the chloro groups in IVb should correspond to those in 2,4,6-trichloro-5-nitropyrimidine (IVc). The chloro group at the 2-position in IVc has been shown[10] to be least reactive. The

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract No. SA-43-ph-3025.

(2) D. E. O'Brien, F. Baiocchi, R. K. Robins, and C. C. Cheng, *J. Med. Pharm. Chem.*, **5**, 1085 (1962).

(3) A. V. Merkatz, *Ber.*, **25**, 749 (1892).

(4) J. T. Thurston, J. R. Dudley, D. W. Kaiser, I. Heckenbleikner, F. C. Shaefer, and D. Holm-Hansen, *J. Am. Chem. Soc.*, **73**, 298 (1951).

(5) C. K. Banks, *ibid.*, **66**, 1127 (1944).

(6) W. Gabriel, *Ber.*, **34**, 3363 (1901).

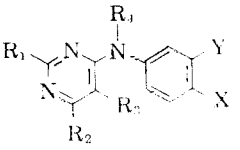
(7) R. M. Cresswell and H. C. S. Wood, *J. Chem. Soc.*, 4768 (1960).

(8) J. Nakazawa and M. Watatani, *Takamine Kenkyusho Nempo*, **12**, 32 (1960).

(9) In the monosubstitution reaction of aniline and IV, it was found that when 2 equiv. rather than 1 equiv. of aniline were used yields as high as 80–90% could be achieved. The excess aniline absorbed hydrogen chloride liberated during the reaction to form the aniline hydrochloride, which was readily removed by washing with water. When the reaction temperature was raised to *ca.* 30°, a mixture of mono- and disubstituted products was obtained.

(10) R. K. Robins, K. L. Dille, and B. E. Christensen, *J. Org. Chem.*, **19**, 930 (1954).

TABLE I



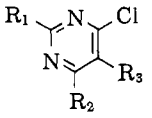
								Calcd.			Found			Ultraviolet absorption, mμ pH 1		pH 11	
R_1	R_2	R_3	R_4	X	Y	Yield, %	M.p., °C.	C	H	N	C	H	N	λ_{max}	$\epsilon \times 10^{-3}$	λ_{max}	$\epsilon \times 10^{-3}$
NH_2	NH_2	C_2H_5	H	Cl	Cl	45	187–189	48.4	4.1	23.5	48.2	4.1	23.5	252	11.9	225	16.3
														300	30.3	299	28.8
NH_2	NH_2	NO_2	H	Br	H	31	240–242	36.9	2.8	25.8	37.3	3.0	25.6	292	27.6	280	27.0
NH_2	NH_2	NO_2	H	I	H	38	278–279	32.1	2.4	22.3	32.7	2.7	21.8	293	25.2	289	25.0
NH_2	NH_2	Br	H	Br	H	60	233–235	33.4	2.5	19.5	33.9	2.5	19.5	250	12.6	250	12.5
														297	25.2	294	23.7
NH_2	NH_2	Br	H	I	H	63	243–245	29.6	2.2	17.3	29.8	2.3	17.7	252	13.8	252	14.2
														299	27.6	297	26.8
NH_2	NH_2	Br	H	Cl	Cl	79	198–199	34.4	2.3	20.1	34.6	2.5	20.2	252	12.6	252	11.9
														299	25.2	297	23.0
Cl	Cl	CN	H	Br	H	83	274–276	38.1	1.5	16.2	38.7	1.6	16.7	309[c]	28.9		
NH_2	NH_2	CN	H	Br	H	70	256–258	43.2	3.0	27.5	43.0	3.4	27.0	280	22.9	228	18.6
																288	30.2
NH_2	NH_2	CN	H	I	H	76	267–268	37.5	2.6	23.9	37.8	3.0	24.2	283	24.0	226	21.5
																292	32.7
NH_2	NH_2	CN	H	Cl	Cl	63	255–256	44.7	2.7	28.4	44.6	2.8	28.6	223	22.2	230	10.6
														252	20.4	256	14.8
														281	20.1	281	13.3
NH_2	NH_2	$CONH_2$	H	Br	H	50	255–256	40.8	3.4	26.0	40.9	3.4	26.4	283	24.0	228	22.6
																293	29.4
NH_2	NH_2	$CONH_2$	H	I	H	56	260–261	35.6	3.0	22.7	35.4	3.0	22.1	285	25.5	229	22.2
																296	32.2
NH_2	NH_2	NO	CH_3	Br	H	46[b]	270 dec.	40.8	3.4	26.0	40.8	3.8	26.2	252	11.0	245	19.1
																317	11.6
NH_2	NH_2	NO	C_4H_9	Br	H	87[c]	212–214 dec.	46.1	4.7	23.0	46.1	4.7	23.0	243	19.8	250	11.6
														302	11.9	328	15.2
NH_2	$NHCH_3$	H	H	Br	H	58[b]	95–96	42.3[d]	4.5	22.4	42.7	4.5	21.8	297	22.9	235	14.1
																258	11.8
																295	22.9
NH_2	$NHCH_3$	NO	H	Br	H	76	251–252 dec.	40.8	3.4	26.0	40.9	3.5	26.3	250	17.1	251	16.8
														335	8.4	325	13.9
														390	6.8		
NH_2	$NHCH_3$	H	H	Cl	Cl	39[b]	235 dec.	45.1[e]	4.1	23.9	45.4	4.1	23.9	258	12.9	260	13.5
														301	28.4	296	27.9
NH_2	$NHCH_3$	NO	H	Cl	Cl	91	250–252 dec.	42.1	3.2	26.8	42.6	3.1	26.1	250	16.3	251	17.9
														332	8.2	330	14.7
NH_2	$NHCH_3$	NO	H	I	H	56[c]	229–231 dec.	35.7	3.0	22.7	35.6	3.3	22.5	251	20.3	253	19.6
														325	8.2	310	14.1
														390	7.4		
NH_2	$NHCH_3$	NO	CH_3	Br	H	72[b]	205–206 dec.	42.7	3.9	24.9	42.7	4.0	24.5	246	12.5	247	15.2
														268	12.1	320	15.2
NH_2	NHC_4H_9	H	H	Br	H	58[b]	83–85	48.7[e]	5.5	20.3	48.6	5.5	20.7	297	23.8	234	15.2
																295	22.5
NH_2	NHC_4H_9	NO	H	Br	H	65	220–221 dec.	46.1	4.7	23.0	46.1	4.9	23.3	250	18.2	270	18.6
														332	8.0	370	16.1
NH_2	NHC_4H_9	NO	H	I	H	32[b]	202 dec.	40.8	4.2	20.4	40.8	4.3	20.3	253	21.1	235	11.5
																270	16.1
NH_2	NHC_4H_9	NO	H	Cl	Cl	72[b]	202 dec.	47.3	4.5	23.6	47.6	4.5	23.8	249	17.4	268	16.7
NH_2	$N(CH_3)_2$	H	H	Br	H	63[b]	186–188	46.7	4.6	22.7	46.7	4.6	22.3	300	26.2	234	17.0
																255	11.2
																297	21.6
NH_2	$N(CH_3)_2$	NO	H	Br	H	67	207–209 dec.	42.7	3.9	24.9	42.6	3.8	24.0	245	15.2	243	18.8
														278	11.8	318	15.5
$NHCH_3$	Cl	H	H	Br	H	76[c]	201–202 dec.	39.8[d]	3.6	16.9	39.6	3.3	16.8	302	20.8	237	18.5
																267	13.6
																307	20.5
$NHCH_3$	NH_2	NO	H	Br	H	44	259–261 dec.	40.8	3.4	26.0	41.1	3.5	25.5	271	11.0	278	10.5
														340	3.2	332	4.5
$N(CH_3)_2$	NH_2	H	H	Br	H	65[b]	195–196	45.5[e]	4.8	22.1	45.8	4.5	22.1	300	28.2	253	20.9
																300	25.9
$N(CH_3)_2$	NH_2	NO	H	Br	H	65	250 dec.	42.7	3.9	24.9	42.8	3.9	24.5	250	15.7	247	16.7
														295	12.1	337	22.8

[a] Determined in ethanol. [b] Prepared by method A (see Experimental). [c] Prepared by method B (see Experimental). [d] Hydrate. [e] Hemihydrate.

possibility of the formation of 2-(substituted-anilino) isomer was also ruled out by means of paper chromatographic study on the final product, II (R = CN, see Experimental).

Ethanolic ammonia converted 2,4-dichloro-6-(substituted-anilino)-5-pyrimidinecarbonitrile at elevated temperature to the desired 2,4-diamino-6-(substituted-anilino)-5-pyrimidinecarbonitrile (II, R = CN). The corresponding 5-carboxamide derivatives (II, R = $CONH_2$) were readily formed when II (R = CN) was treated with concentrated sulfuric acid at room temperature.

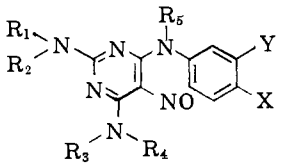
The second area of this study involves the substitution of hydrogen atoms in the primary and secondary



IV

a, $R_1, R_2 = NH_2, R_3 = Br$
b, $R_1, R_2 = Cl, R_3 = CN$
c, $R_1, R_2 = Cl, R_3 = NO_2$
d, $R_1 = NH_2, R_2 = Cl, R_3 = H$
e, $R_1 = N(CH_3)_2, R_2 = NH_2, R_3 = H$
f, $R_1 = NHCH_3, R_2 = Cl, R_3 = H$

amino functions in I by alkyl groups. A general structure of this type of compound is represented by V.



V

Action of a primary or secondary amine with 2-amino-4,6-dichloropyrimidine[11] (IVd) gave 2-amino-4-chloro-6-(substituted-amino)pyrimidine, which was then fused with the appropriate aniline to form 2-amino-4-(substituted-amino)-6-(substituted-anilino)pyrimidine. The resulting product was readily nitrosated to give the corresponding 5-nitroso-6-(substituted-amino)pyrimidine (V, R_1, R_2, R_5 = H). The 5-nitroso-4-(N-substituted-anilino)pyrimidines (V, R_1, R_2, R_3, R_4 = H) were prepared by the reaction of *p*-bromo-N-substituted aniline and diamino-6-chloropyrimidine,[6] followed by nitrosation.

6-Amino-4-chloro-2-dimethylaminopyrimidine (IVe), prepared by Langerman and Banks,[12] was treated with substituted aniline to give 4-amino-2-dimethylamino-6-(substituted-anilino)pyrimidine. Nitrosation afforded the 2-dimethylamino analog of I (V, R_1, R_2 = CH_3; R_3, R_4, R_5 = H).

Although the reaction of methylguanidine with diethyl 2,2-diethylmalonate gave exclusively one product (the 2-methylaminopyrimidine derivative),[13] a mixture of two pyrimidines (2-methylamino-4,6-dihydroxypyrimidine and the isomeric 2-amino-1-methyl derivative) was obtained from the reaction of methylguanidine and diethyl malonate.[14] The major product, 4,6-dihydroxy-2-methylaminopyrimidine, was treated with phosphorus oxychloride, to yield 4,6-dichloro-2-methylaminopyrimidine (IVf).[14] This product was found to be identical with that prepared from 4,6-dichloro-2-methylsulfonylpyrimidine[15] and methylamine. The desired 2-methylaminopyrimidine analog of I was then synthesized from IVf by standard preparative procedures.

Preliminary antitumor screening results[16] of these derivatives are listed in Table II. With the information presently available it appears that: (1) unlike most pyrimidine and purine derivatives, pyrimidines of this type are inactive against the Le-1210 system; (2) replacement of the nitroso group by an ethyl group results in the loss of the original activity; (3) activity in Ca-755 decreased with the reduction of the 5-nitroso group to the 5-amino group; (4) the corresponding *s*-triazine derivatives showed no activity; (5) replacement of the 5-nitroso group by a bromine atom caused appreciable loss of activity in Ca-755; (6) the corresponding 5-nitro and 5-carbamoyl analogs possess no activity in the Ca-755 system; (7) activity in the Sa-180 system was slightly improved with the replacement of the nitroso by a cyano moiety; and (8) substitution of the hydrogen atoms in the primary and secondary amino groups (structure V) by small alkyl groups (*e.g.*, methyl) resulted in comparatively lower toxicity with retention of original activity.

Experimental[17]

2,4-Diamino-6-(*p*-bromoanilino)-*s*-triazine (III) (X = Br, Y = H).—A solution of 2,4-diamino-6-chloro-*s*-triazine (14.6 g., 0.1 mole),[4] *p*-bromoaniline (18.9 g., 0.11 mole), concentrated hydrochloric acid (1 ml.), and 33% aqueous ethanol (300 ml.) was refluxed for 4 hr. The reaction mixture was then added to 1 l. of boiling water, followed by the addition of enough concentrated hydrochloric acid to effect complete solution. The solution was treated with charcoal and filtered, and the filtrate adjusted with aqueous ammonia to pH 8. The precipitated product was filtered, washed with ether, and dried at 100° to give 21 g. (70%) of the product, m.p. 251–253°. For analysis a small sample was recrystallized from butanol and dried at 110°, m.p. 253–254°; $\lambda_{max}^{pH\ 1}$ 268 mμ (ε 23,300); $\lambda_{max}^{pH\ 11}$ 265 mμ (ε 27,800).

Anal. Calcd. for $C_9H_9BrN_6 \cdot H_2O$: C, 36.1; H, 3.7; N, 28.1. Found: C, 36.4; H, 3.7; N, 28.3.

2,4-Diamino-6-(3,4-dichloroanilino)-*s*-triazine (III) (X, Y = Cl) was similarly prepared in 66% yield, m.p. 202–204°; $\lambda_{max}^{pH\ 1}$ 267 mμ (ε 23,500); $\lambda_{max}^{pH\ 11}$ 265 mμ (ε 27,400).

Anal. Calcd. for $C_9H_8Cl_2N_6 \cdot {}^1/_2H_2O$: C, 38.6; H, 3.2; N, 30.0 Found: C, 38.9; H, 3.0; N, 30.0.

2,4-Diamino-6-(3,4-dichloroanilino)-5-ethylpyrimidine (II) (R = C_2H_5; X, Y = Cl).—A mixture of 2,4-diamino-6-chloro-5-ethylpyrimidine[3] (17.3 g., 0.1 mole), 3,4-dichloroaniline (17.8 g., 0.11 mole), and glacial acetic acid (6 g., 0.1 mole) was fused at 180° for 20 min. The resultant glassy substance was dissolved in 200 ml. of dilute hydrochloric acid, treated with charcoal, and filtered. The filtrate was neutralized with aqueous ammonia and cooled. The precipitated product was then filtered, washed well with ether, recrystallized from aqueous ethanol, and dried at 100° (see Table I).

2,4-Diamino-6-chloro-5-nitropyrimidine.—To a mixture of concentrated sulfuric acid (90 ml.) and fuming nitric acid (90 ml., sp. gr. 1.60 at 15°) cooled at 20° was slowly added, with vigorous stirring, 2,4-diamino-6-chloropyrimidine (58 g., 0.4 mole).[6] The temperature during the addition was maintained between 20 and 25° by outside cooling. After the addition was complete, the solution was stirred at room temperature for 1 hr. The yellow solution was then added at once to 750 g. of flaked ice, and the suspension allowed to stand for 1 hr. The resulting yellow solid was filtered and washed with water until all the acid was removed. The solid was then purified by extraction with 500 ml. of boiling water, washed with 200 ml. of ethanol, and dried at 100° to give 22 g. (29%) of analytically pure product, m.p. 228° with violent decomposition; $\lambda_{max}^{pH\ 1}$ 298 mμ (ε 11,700), 364 mμ (ε 5500); $\lambda_{max}^{pH\ 11}$ 285 mμ (ε 8300), 311 mμ (ε 7400).

Anal. Calcd. for $C_4H_4ClN_5O_2$: C, 25.3; H, 2.1; N, 36.9. Found: C, 25.4; H, 2.3; N, 36.7.

2,4-Diamino-5-nitro-6-(substituted-anilino)pyrimidines (Table I). General Procedure.—A mixture of 2,4-diamino-6-chloro-5-

(11) (a) H. Büttner, *Ber.*, **36**, 2232 (1903); (b) W. Gabriel and B. Coleman, *ibid.*, **36**, 3381 (1903).

(12) M. J. Langerman and C. K. Banks, *J. Am. Chem. Soc.*, **73**, 3011 (1951).

(13) (a) R. Majima, *Ber.*, **41**, 176 (1908); (b) B. Roth, J. M. Smith, and M. E. Hultquist, *J. Am. Chem. Soc.*, **73**, 2864 (1951).

(14) (a) W. Winkelmann, *J. prakt. Chem.*, **115**, 292 (1927); (b) W. R. Boon, *J. Chem. Soc.*, 2146 (1957).

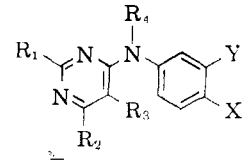
(15) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 792 (1961).

(16) The biological testing was performed by Battelle Memorial Institute, Microbiological Associates, Inc., and Wisconsin Alumni Research Foundation under the auspices of the Cancer Chemotherapy National Service Center.

(17) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer.

Table II

Comparison of Antitumor Activities of 2,4-Diamino-6-(halogenoanilino)-5-nitrosopyrimidines and Related Compounds



R_1	R_2	R_3	R_4	X	Y	Dose[a] mg./kg.	Activity[b] Sa-180	Activity[b] Ca-755
NH_2	NH_2	NO	H	Br	H	44	−	++
NH_2	NH_2	NO	H	I	H	32	±	++
NH_2	NH_2	NO	H	Cl	Cl	57	±	++
NH_2	NH_2	[c]	H	Br	H	350	−	−
NH_2	NH_2	[c]	H	Cl	Cl	63	−	−
NH_2	NH_2	[c]	H	Br	Br	63	−	−
NH_2	NH_2	C_2H_5	H	CH_3	H	6	−	−
NH_2	NH_2	C_2H_5	H	CH_3	CH_3	50	−	−
NH_2	NH_2	C_2H_5	H	Cl	Cl	50	−	−
NH_2	NH_2	NH_2	H	CH_3	H	120	−	−
NH_2	NH_2	NH_2	H	CH_3	CH_3	250	−	−
NH_2	NH_2	NH_2	H	CH_3	Cl	100	−	−
NH_2	NH_2	NH_2	H	Br	H	31	−	+
NH_2	NH_2	Br	H	Br	H	500	−	−
NH_2	NH_2	Br	H	I	H	400	−	±
NH_2	NH_2	Br	H	Cl	Cl	200	−	±
NH_2	NH_2	NO_2	H	Br	H	125	−	−
NH_2	NH_2	CN	H	Br	H	200	−	−
NH_2	NH_2	CN	H	I	H	250	±	−
NH_2	NH_2	CN	H	Cl	Cl	125	±	+
NH_2	NH_2	$CONH_2$	H	Br	H	125	−	−
NH_2	NH_2	$CONH_2$	H	I	H	250	−	−
NH_2	$NHCH_3$	NO	H	Br	H	400	+	++
NH_2	$NH(CH_2)_3CH_3$	NO	H	Br	H	400	−	−
NH_2	$NHCH_3$	NO	H	I	H	200	−	++
NH_2	$NH(CH_2)_3CH_3$	NO	H	I	H	200	−	+
NH_2	$NHCH_3$	NO	H	Cl	Cl	400	+	+
NH_2	$NH(CH_2)_3CH_3$	NO	H	Cl	Cl	400	−	±
NH_2	$N(CH_3)_2$	NO	H	Br	H	200	−	++
$NHCH_3$	NH_2	NO	H	Br	H	200	−	++
$N(CH_3)_2$	NH_2	NO	H	Br	H	200	−	+
NH_2	NH_2	NO	CH_3	Br	H	400	−	−
NH_2	NH_2	NO	$(CH_2)_3CH_3$	Br	H	400	−	−
NH_2	$NHCH_3$	NO	CH_3	Br	H	400	+	++

[a] Below toxicity level. [b] ++, activity confirmed; +, activity not yet confirmed; ±, borderline activity; −, inactive. Testing done by the Contract Screeners of CCNSC, see ref. 16. [c] Ring nitrogen (*s*-triazine).

nitropyrimidine (18.9 g., 0.1 mole), a substituted aniline (0.1 mole), and dimethylformamide (160 ml.) was heated at 120° with stirring for 16 hr. The dark solution was then added to 300 ml. of boiling water followed by the addition of enough dimethylformamide to effect complete solution. The boiling solution was treated with charcoal, filtered, and the filtrate allowed to cool slowly. The yellow-orange crystalline product was filtered, washed with water, ethanol, and ether, and recrystallized from aqueous dimethylformamide.

2,4-Diamino-5-bromo-6-chloropyrimidine.—A solution of 2,4-diamino-6-chloropyrimidine (28.9 g., 0.2 mole)[6] and freshly fused sodium acetate (16.4 g., 0.2 mole) in acetic acid (300 ml.) was warmed to 90°. To the hot, stirred solution was added dropwise 32 g. (0.2 mole) of bromine. After the addition was complete, the mixture was stirred and heated at 90–95° for 1 hr. A small amount of solid was separated by filtration. The filtrate was cooled, the resulting crystalline product was filtered, washed with three 100-ml. portions of ether, and dried at 100° to yield 39 g. (88%), m.p. 218–219°. Recrystallization from ethanol and drying at 130° raised the melting point to 219–220°; $\lambda_{max}^{ethanol}$ 232 mμ (ε 12,300), 293 mμ (ε 7800).

Anal. Calcd. for $C_4H_4BrClN_4$: C, 21.5; H, 1.8; N, 25.0. Found: C, 21.9; H, 2.0; N, 25.2.

2,4-Diamino-5-bromo-6-(substituted-anilino)pyrimidines (Table I). General Procedure.—A solution of 2,4-diamino-5-bromo-6-chloropyrimidine (22.3 g., 0.1 mole), a substituted aniline (0.1 mole), 1 ml. of concentrated hydrochloric acid, and 300 ml. of 33% aqueous ethanol was refluxed for 6 hr. The reaction mixture was then added to 1 l. of boiling water, and enough concentrated hydrochloric acid was added to effect complete solution. The solution was treated with charcoal and filtered. The filtrate was adjusted to pH 8 by means of aqueous ammonia. The resulting precipitated product was filtered while hot, washed with two 250-ml. portions of boiling ethanol, and recrystallized from a mixture of water and dimethylformamide.

2,4-Dichloro-6-(substituted-anilino)-5-pyrimidinecarbonitriles (Table I). General Procedure.—To a cooled (0°) solution of 2,4,6-trichloro-5-pyrimidinecarbonitrile[7] (20.8 g., 0.1 mole) in 500 ml. of anhydrous ether was slowly added a solution of a substituted aniline (0.2 mole) in a minimum amount of anhydrous ether. The temperature of the solution during the addition did not exceed 5°. After the addition was complete, the mixture was stirred at 0–5° for 3 hr. The solid product was filtered, washed with two 100-ml. portions of ether, three 250-ml. portions of water (which removed the substituted aniline hydrochloride), and again with two 100 ml. portions of ether, and air dried. The product is sufficiently pure to be carried on to the next step. Purification for analysis can be accomplished by recrystallization from ethanol.

2,4-Diamino-6-(substituted-anilino)-5-pyrimidinecarbonitriles (Table I). General Procedure.—A mixture of 2,4-dichloro-6-(substituted-anilino)-5-pyrimidinecarbonitrile (0.05 mole) and

250 ml. of saturated ethanolic ammonia was heated in an autoclave at 135° for 12 hr. The resulting solution was evaporated to dryness and the residue recrystallized from butanol. A final recrystallization from aqueous ethanol afforded an analytically pure product.

The structural assignment (see Discussion part) in this group of compounds was further substantiated by paper chromatographic measurements (one spot in each case). R_f values for 2,4-diamino-6-(*p*-bromoanilino)-5-pyrimidinecarbonitrile: 0.71 (isopropyl alcohol–water, 7:3), and 0.80 (butanol saturated with acetic acid); for 2,4-diamino-6-(*p*-iodoanilino)-5-pyrimidinecarbonitrile: 0.73 and 0.81, respectively.

2,4-Diamino-6-(substituted-anilino)-5-pyrimidinecarboxamide (Table I). General Procedure.—With good stirring, 2,4-diamino-6-(substituted-anilino)-5-pyrimidinecarbonitrile (0.05 mole) was added slowly to 50 ml. of concentrated sulfuric acid at 30–35°. The solution was stirred at this temperature for 12 hr. and then added, with stirring, to 500 g. of flaked ice. The precipitated product was separated by filtration and washed well with ice–water. The crude product was dissolved in hot, dilute sulfuric acid, treated with charcoal, and filtered. The filtrate was adjusted to pH 8 with aqueous ammonia and the product filtered while hot, washed with water, and dried at 100°. Repeated reprecipitation afforded product of analytical purity.

4,6-Dichloro-2-methylaminopyrimidine.—In a flask equipped with two modified Friedrichs condensers designed to retain low boiling liquids was added 23 g. (0.23 mole) of triethylamine and 7 g. (0.23 mole) of methylamine diluted with 200 ml. of ethyl acetate. To this solution was added 30 g. (0.154 mole) of 4,6-dichloro-2-(methylsulfonyl)pyrimidine[15] dissolved in 200 ml. of ethyl acetate. The temperature was kept below 35° during the entire reaction. After 2 hr. of stirring the reaction mixture was evaporated and the pale yellow residue recrystallized from ethanol to give 13 g. (77%) of white crystals, m.p. 162–163°. The product was found to be identical with that reported by Winkelmann[14a] and Boon.[14b]

4-Amino-6-chloro-2-methylaminopyrimidine.—A mixture of 4,6-dichloro-2-methylaminopyrimidine (10.8 g.) and ethanolic ammonia was heated at 80° for 8 hr. in a sealed vessel. Evaporation of the reaction mixture and purification of the product from methanol afforded 7 g. (65%) of white crystals, m.p. 193–194°.

Anal. Calcd. for $C_5H_7ClN_4 \cdot H_2O$: N, 31.8. Found: N, 31.9.

General Preparations of 4-(Substituted-anilino)-5-nitrosopyrimidines (V). A.—A mixture of the appropriate 4-chloropyrimidine, an equivalent amount (plus a 10% excess) of the substituted aniline, and several milliliters of concentrated hydrochloric acid was heated in an oil bath. A clear, dark colored melt was observed around 150° followed by an exothermic reaction that caused the temperature to rise to *ca.* 200°. The reaction mixture was held at this temperature for several minutes. It was then cooled, dissolved in boiling ethanol, treated with charcoal, and filtered. The filtrate was made basic with dilute aqueous ammonia and cooled. The precipitated 4-(substituted-anilino)pyrimidine was filtered and washed with water. Nitrosation of the intermediate to the desired product was carried out by the procedure of O'Brien, *et al.*[2]

B.—A solution of 0.10 mole of the appropriate 4-chloropyrimidine, 0.11 mole of the substituted aniline, and 2 ml. of concentrated hydrochloric acid in 200 ml. of 50% ethanol was refluxed for 4 hr. The reaction mixture was poured into 800 ml. of boiling water. The resulting solution was treated with charcoal, filtered, and the filtrate adjusted to pH 8 with aqueous ammonia. On cooling it deposited the intermediate 4-(substituted-anilino)-pyrimidine, which was then isolated and nitrosated as described in Method A.

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Synthesis and Antitumor Activity of 9-(Tetrahydro-2-furyl)purine Analogs of Biologically Important Deoxynucleosides[1]

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The syntheses of the 9-(tetrahydro-2-furyl) derivatives of hypoxanthine, guanine, and 2-amino-6-purinethiol (6-thioguanine) have been accomplished. The reaction of 2,3-dihydro-2-methylfuran with 6-chloropurine has been studied. Several of the 9-(tetrahydro-2-furyl)purines exhibit significant antitumor activity against a variety of experimental mouse tumors. The significance of these results is discussed in terms of therapeutic index, transport, and structural relationship to various purine-2′-deoxynucleosides and other biologically active purine derivatives.

The synthesis of certain 6-substituted-9-(tetrahydro-2-pyranyl)purines and 6-substituted-9-(tetrahydro-2-furyl)purines has recently been reported.[2,3] The preliminary antitumor activity of several of these derivatives has prompted a detailed study of this group of compounds. Since the 9-(tetrahydro-2-furyl)purines appeared to be superior in the early stages of animal testing, attention was directed to these derivatives. Because the 9-(tetrahydro-2-furyl)purines can be envisaged as analogs of the important naturally occurring purine deoxynucleosides, the first major goal of this work was to synthesize the 9-(tetrahydro-2-furyl) analog of 2′-deoxyguanosine and 2′-deoxyinosine. 6-Amino-9-(tetrahydro-2-furyl)purine, the analog of 2′-deoxyadenosine, had previously been prepared[3] and exhibited definite antitumor activity. An attempt to prepare 6-hydroxy-9-(tetrahydro-2-furyl)purine (IV) directly from 6-chloro-9-(tetrahydro-2-furyl)purine with aqueous sodium hydroxide resulted in degradation of the purine ring to give 6-chloro-4,5-diaminopyrimidine[4] as the only isolatable product. In order to obtain IV, 6-benzyloxypurine (I) was prepared from 6-chloropurine by the general method of Huber.[5] Treatment of I with 2,3-dihydrofuran in the presence of acid gave

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