Synthesis of 6-(N-Alkyl-N-arylamino)pyrimidines As Potential Antimetabolites[1]

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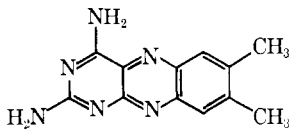
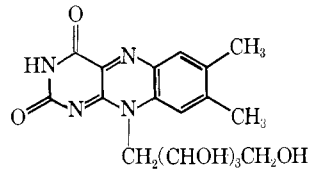
Thirty-eight new 6-(N-alkyl-N-arylamino)pyrimidines were prepared as open-chain analogs of riboflavin that lack the N_{10} atom of the central ring. Most of these compounds were prepared by simple coupling reaction between the appropriate 2,4-disubstituted 6-chloropyrimidine and an N-alkylaniline, usually under fusion conditions, except in the case of the 5-NO_2 derivatives where Cl was sufficiently activated to react in refluxing EtOH, Et_3N, or DMF. Several of the compounds showed *in vitro* activity against Sarcoma 180 cells, 2,4-diamino-6-(N-ethyl-3,4-dimethylanilino)pyrimidine·HCl (**34**) being the most active.

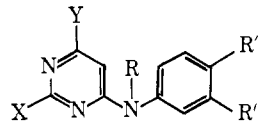
2,4-Diamino-6,7-dimethyl-2,4-dideoxyalloxazine (I) has been found to antagonize both riboflavin (II) and folinic acid in several microbiological test systems[5,6] and to inhibit the growth of transplanted tumors in mice.[7,8] However, the poor tissue absorption properties of I limited further *in vivo* studies with this compound. It was felt that introduction of various alkyl and hydroxyalkyl chains in the N_9 position of I might lead to compounds with increased solubility and better absorption properties.[9] Such compounds would also bear a greater structural resemblance to II. However, direct alkylation of I resulted in substitution in the N_1 position,[9] and our attempts to react *dimeric* 4,5-dimethyl-*o*-benzoquinone[5] with a variety of 4-substituted 6-alkylamino-2,5-diaminopyrimidines[10] led to condensation products which were difficult to purify.



I



II



III

Cheng, O'Brien, and coworkers reported the antitumor activities of various 5-nitro- (and 5-nitroso)-2,4-diamino-6-arylaminopyrimidines, and noted the structural resemblance of 6-arylaminopyrimidines to riboflavin.[11,12] It has also been observed that a mammalian flavokinase was inhibited *in vitro* by a homologous series of 9-(ω-hydroxyalkyl)-6,7-dimethylisoalloxazines, and it appeared that the alkyl chain at the 9 position of the heterocyclic ring system contributed to the binding of the inhibitor to the enzyme.[13] Thus, we were encouraged to prepare a variety of 2,4-disubstituted 6-(N-alkyl-N-arylamino)pyrimidines (III, R = alkyl; R′ = H or CH_3), including some which were substituted in the 5 position of the pyrimidine moiety with a nitro or nitroso group.

While 6-(N-alkyl-3,4-dimethylanilino)pyrimidines (III, R = alkyl; R′ = CH_3) would bear the greatest structural resemblance to riboflavin (II), several 6-(N-alkylanilino)pyrimidines (III, R = alkyl; R′ = H) were also prepared, making use of the commercial availability of N-benzyl-, N-ethyl-, and N-methylanilines. The reaction of 2 mol of 3,4-dimethylaniline with 1 mol of the appropriate acyl chloride gave a series of 3′,4′-dimethylanilides (IV, Scheme I; **1–5**, Table I) which were reduced by the method of Brown and Heim[14] in good yield to the corresponding N-alkyl-3,4-dimethylanilines (V, R′ = CH_3, Scheme I; **6–10**, Table II). By treating 2-amino-4,6-dichloropyrimidine with the N-alkyl-N-arylamines in a fusion reaction similar to that described by O'Brien and coworkers,[15] a series of 2-amino-4-chloro-6-(N-alkyl-N-arylamino)pyrimidines (VI, Scheme I; **11–18**, Table III) was obtained. Contrary to previous reports of similar condensations,[15,16] we found the addition of mineral acid to be unnecessary. The 4-chloro derivatives were readily converted into the 4-hydroxy analogs (VII, Scheme I; **19–26**, Table III) by treating the former with powdered NaOH in ethylene glycol at elevated temperatures.[15]

A series of 6-(N-alkyl-N-arylamino)uracils (IX, Scheme II; **27–32**, Table III) were prepared by fusing 6-chlorouracil[17] (VIII) with the N-alkyl-N-arylamines. Similarly, we synthesized a series of 2,4-diamino-6-(N-alkyl-N-arylamino)pyrimidines (XI, Scheme II;

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(4) To whom communications should be directed.

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(12) D. E. O'Brien, C. C. Cheng, and W. Pfleiderer, *J. Med. Chem.*, **9**, 573 (1966).

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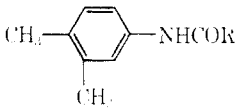
TABLE I
3′,4′-DIMETHYLANILIDES



No.	R	Yield, %[a]	Recrystn solvent	Mp, °C	Formula[b]
1	Me	98.1	EtOH–H_2O	97–98[c]	
2	Et	96.3	EtOH–H_2O	105.5–106.5	$C_{11}H_{15}NO$
3	Pr	95.8	Heptane	71.5–72.5	$C_{12}H_{17}NO$
4	Ph	91.8	EtOH–H_2O	137–138.5	$C_{15}H_{15}NO$
5	CH_2Ph	96.0	C_6H_6	122.5–124	$C_{16}H_{17}NO$

[a] Recrystallized product. [b] Satisfactory analysis for C, H, N were obtained. [c] Reported mp 98°, "Dictionary of Organic Compounds" Sir Jan Heilbron *et al.*, Ed., Eyre and Spotteswoode, London, 4th ed, 1965, Vol. II, p 1143.

SCHEME I

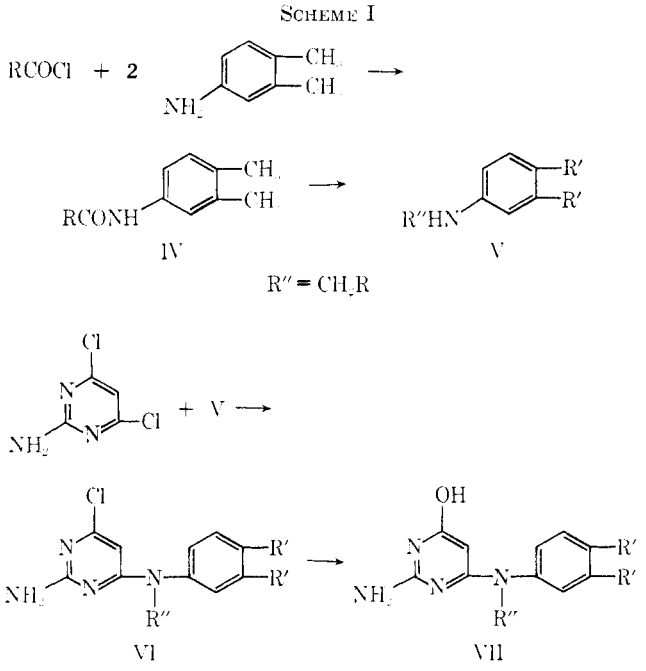
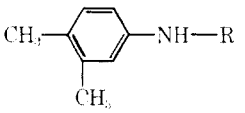


TABLE II
N-ALKYL-3,4-DIMETHYLANILINES



No.	R	Yield, %	Bp, °C (mm)	Formula[a]
6	Et	94.6	56–58 (0.1)	$C_{10}H_{15}N$
7	Pr	88.9	94–96 (1.5)	$C_{11}H_{17}N$
8	Bu	90.8	108–110 (1.5)	$C_{12}H_{19}N$
9[b]	CH_2Ph	81.6	139–142 (0.2)	$C_{15}H_{17}N$
10	CH_2CH_2Ph	84.8	141–143 (0.5)	$C_{16}H_{19}N$

[a] Satisfactory analysis were obtained for C, H, N. [b] Prepared by the addition of approximately 2.4 mol of THF-borane to a suspension of 0.175 mol of amide in 160 ml of THF; reflux time: 7 hr.

33–37, Table III) by treating 6-chloro-2,4-diaminopyrimidine (X) with the *N*-alkyl-*N*-arylamines. The 2,4-diamino-6-(*N*-alkyl-3,4-dimethylanilino)pyrimidines were isolated as the HCl salts, since members of the series could not be crystallized as the free base.

By refluxing an EtOH solution of 6-chloro-5-nitrouracil[17] (XII) and the appropriate *N*-alkyl-*N*-arylamine in the presence of Et_3N, a series of 6-(*N*-alkyl-*N*-arylamino)-5-nitrouracils (XIII, Scheme II; **38–42**, Table IV) was readily prepared. The preparation of several 2,4-diamino-6-(*N*-alkylanilino)-5-nitropyrimidines (XV, Scheme II; **43–44**, Table IV) was accomplished by refluxing 6-chloro-2,4-diamino-5-nitropyrimidine[12] (XIV) with an excess of the *N*-alkyl-*N*-arylamine in DMF. By treating a solution of **34** in dilute HCl with $NaNO_2$, 2,4-diamino-6-(*N*-ethyl-3,4-dimethylanilino)-5-nitrosopyrimidine (**50**) was obtained.

Biological Testing.—When screened against a Sarcoma-180 cell line in tissue culture,[18] **14**, **17**, **19**, and **33** displayed moderate activity (ID_{50} = 3–6 μg/ml) while 2,4-diamino-6-(*N*-ethyl-3,4-dimethylanilino)pyrimidine·HCl (**34**) showed marked activity (ID_{50} = 0.6 μg/ml). None of the compounds inhibited the growth of *Lactobacillus leichmannii*[19] at concentrations of 50–200 μg/ml. Only the diamino derivatives **33**, **34**, **35**, and **50** showed significant *in vivo* toxicity (LD_{50} 20–80 mg/kg, by subcutaneous route) in BDF male mice. Two of the 6-(*N*-alkyl-*N*-aryl)pyrimidines, **14** and **47**, displayed moderate antitumor activity against adenocarcinoma 755 in mice. The other compounds tested were inactive in this test system as well as against Ehrlich ascites in mice. Compound **34** was also tested against Walker carcinosarcoma 256 in the rat, but it showed no activity in this tumor test.

Experimental Section[20]

N-Benzylaniline, *N*-ethylaniline, and *N*-methylaniline were purchased from Distillation Products Industries, Rochester, N. Y., AcCl and BzCl from the J. T. Baker Chemical Co., Phillipsburg, N. J., borane–THF solution from Metal Hydrides, Inc., Beverly, Mass., and 2-amino-4,6-dichloropyrimidine, 6-chloro-2,4-diaminopyrimidine, 3,4-dimethylaniline, as well as PrCOCl, $PhCH_2COCl$, and EtCOCl from the Aldrich Chemical Co., Inc., Milwaukee, Wis.

3′,4′-Dimethylanilides (1–5, Table I).—A solution of the acyl chloride (0.1 mol) in C_6H_6 (50 ml) was added dropwise with stirring to a solution of 3,4-dimethylaniline (0.2 mol) in C_6H_6

(18) N. Back, R. R. Sidebs, and A. E. Mahsun, *Antibiot. Chemother.* (Washington, D. C.), **11**, 652 (1961).

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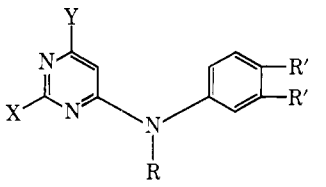
(20) The crude products were recrystallized from the solvents indicated in the tables. Melting points were determined in a Mel-Temp apparatus and are uncorrected. The ultraviolet spectra were determined on a Perkin-Elmer Model 202 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and were within ±0.4% of the theoretical values.

Table III

6-(*N*-Alkyl-*N*-aryl)aminopyrimidines



No.	X	Y	R	R	Yield, %[a]	Recrystn solvent	Mp, °C	Formula[b]
11	NH_2	Cl	CH_3	H	78.0	EtOAc–cyclohexane	176–178[c]	
12	NH_2	Cl	C_2H_5	H	72.6	Cyclohexane	155–156	$C_{19}H_{14}ClN_4$
13	NH_2	Cl	$C_6H_5CH_2$	H	84.6	EtOH, H_2O	166–168	$C_{17}H_{15}ClN_4$
14	NH_2	Cl	C_2H_5	CH_3	67.2	EtOAc	179–181	$C_{14}H_{17}ClN_4$
15	NH_2	Cl	*n*-C_3H_7	CH_3	58.1	Heptane	157–158.5	$C_{15}H_{19}ClN_4$
16	NH_2	Cl	*n*-C_4H_9	CH_3	73.2	Heptane	157–158	$C_{16}H_{21}ClN_4$
17	NH_2	Cl	$C_6H_5CH_2$	CH_3	49.3	EtOAc	212.5–214	$C_{11}H_{19}ClN_4$
18	NH_2	Cl	$C_6H_5CH_2CH_2$	CH_3	60.8	Heptane	200–202	$C_{20}H_{21}ClN_4$
19	NH_2	OH	CH_3	H	67.1	MeOH	274–275.5	$C_{11}H_{12}N_4O$
20	NH_2	OH	C_2H_5	H	84.0	EtOH	275–276	$C_{12}H_{14}N_4O$
21	NH_2	OH	$C_6H_5CH_2$	H	88.7	MeOH	248.5–250	$C_{17}H_{16}N_4O$
22	NH_2	OH	C_2H_5	CH_3	58.1	MeOH	255–257	$C_{14}H_{18}N_4O$
23	NH_2	OH	*n*-C_3H_7	CH_3	53.0	MeOH–H_2O	245–246.5	$C_{15}H_{20}N_4O$
24	NH_2	OH	*n*-C_4H_9	CH_3	43.7	MeOH	239.5–241.5	$C_{16}H_{22}N_4O$
25	NH_2	OH	$C_6H_5CH_2$	CH_3	42.2	EtOH	241.5–243.5	$C_{19}H_{20}N_4O$
26	NH_2	OH	$C_6H_5CH_2CH_2$	CH_3	58.7	EtAc	218–220	$C_{20}H_{22}N_4O$
27[d]	OH	OH	CH_3	H	98.1	MeOH	285–287 dec	$C_{11}H_{11}N_3O_2$
28	OH	OH	C_2H_5	H	83.4	DMF–MeOH	284.5–286	$C_{12}H_{13}N_3O_2$
29	OH	OH	C_2H_5	CH_3	71.8	EtOH–H_2O	269–270 dec	$C_{14}H_{17}N_3O_2$
30	OH	OH	*n*-C_3H_7	CH_3	76.9	EtOH	235–237	$C_{15}H_{19}N_3O_2$
31	OH	OH	*n*-C_4H_9	CH_3	84.7	EtOH	251.5–253.5	$C_{16}H_{21}N_3O_2$
32	OH	OH	$C_6H_5CH_2$	CH_3	58.7	EtOH	240.5–242	$C_{20}H_{21}N_3O_2$
33[e]	NH_2	NH_2	C_2H_5	H	83.0	EtOH–H_2O	157–158	$C_{12}H_{15}N_5$
34[e]	NH_2	NH_2	C_2H_5	CH_3	70.4	C_6H_6	262.5–263.5	$C_{14}H_{20}ClN_5$
35[e]	NH_2	NH_2	*n*-C_3H_7	CH_3	78.3	Acetone–EtOAc	203.5–205.5	$C_{15}H_{22}ClN_5$
36[e]	NH_2	NH_2	*n*-C_4H_9	CH_3	37.8	EtOH–EtOAc	163–165	$C_{16}H_{24}ClN_5$
37	NH_2	NH_2	$C_6H_5CH_2CH_2$	CH_3	86.6	MeOH–EtOAc–Heptane	149.5–152.5	$C_{20}H_{24}ClN_5$

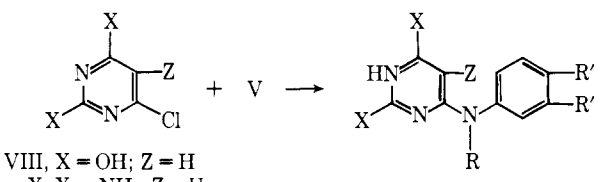
[a] Recrystallized product. [b] Except for compound 11 which was not analyzed, satisfactory analysis were obtained for C, H, and N. [c] Reported[15] mp: 177–178°. [d] B. R. Baker and W. Rzeszotarski, *J. Med. Chem.*, **10**, 1109 (1967) report mp 287–289° for this compound. [e] As the HCl salt.

(500 ml). The reaction mixture was then briefly heated at reflux and filtered to remove the amine·HCl. The salt was washed with hot C_6H_6 (50 ml), the filtrate and washings were combined, and the C_6H_6 was removed by heating *in vacuo* on the steam bath. The crude amide was dissolved in a minimum amount of hot EtOH and poured with stirring into cold H_2O (500 ml); the crude product was separated by filtration, washed with cold H_2O, and dried.

***N*-Alkyl-3,4-dimethylanilines (6–10, Table II).**—Essentially the procedure of Brown and Heim[14] was followed. A solution of the amide (0.175 mol) in a minimum amount of freshly distilled THF was added dropwise with stirring to the cold borane–THF solution (375 ml, 1*M*), in the course of 0.5–0.75 hr (N_2 atm). The mixture was refluxed for 2 hr, followed by cooling to 5–10°. HCl (6 *N*, 50 ml) was added to the cold, stirred reaction mixture (water-cooled condenser) *cautiously*, followed by the rapid addition of H_2O (50 ml). After removal of the THF by distillation, hot H_2O was added (100–300 ml), then the mixture was cooled and made strongly basic with NaOH. The crude amine was extracted into three 100-ml portions of Et_2O. After drying (Na_2SO_4), Et_2O was removed *in vacuo* and the product purified by distillation.

2-Amino-4-chloro-6-(*N*-alkyl-*N*-arylamino)pyrimidines (11–18, Table III).—A mixture of the *N*-alkyl-*N*-arylamine (0.02 mol) and 2-amino-4,6-dichloropyrimidine (0.02 mol) was placed in a 2.5 × 10 cm test tube which was then partially immersed in a bath preheated to 195°. The reaction mixture was stirred until solution occurred, followed by a strong exothermic reaction in which HCl gas was evolved. Heating was continued for 0.5 hr, and sufficient hot ethylene glycol was added to dissolve the yellow oil. The hot solution was poured with vigorous stirring into dilute NH_4OH (400 ml). After standing overnight, the crude solid was collected by filtration, washed with H_2O, and dried.

Scheme II



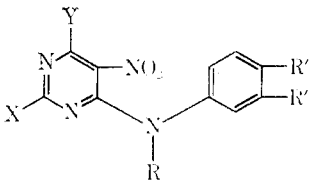
VIII, X = OH; Z = H
X, X = NH_2; Z = H
XII, X = OH; Z = NO_2
XIV, X = NH_2; Z = NO_2

IX, X = OH; Z = H
XI, X = NH_2; Z = H
XIII, X = OH; Z = NO_2
XV, X = NH_2; Z = NO_2

2-Amino-6-(*N*-alkyl-*N*-arylamino)-4-pyrimidinols (19–26, Table III).—A mixture of the chloropyrimidine (0.01 mol), powdered NaOH (2.0 g), and ethylene glycol (30 ml) was heated (as above) at 165°. The reaction was stirred occasionally until solution was effected. (If some of the chloropyrimidine was undissolved after heating 0.5 hr, sufficient additional hot glycol was added to dissolve the compound.) Heating was continued for a total of 1.5 hr, and the hot reaction mixture was poured, with vigorous stirring, into H_2O (250 ml). The filtrate was adjusted

TABLE IV

6-(*N*-ALKYL-*N*-ARYL)AMINO-5-NITROPYRIMIDINES



No.	X	R	R′	Yield, %[a]	Recrystn solvent	Mp, °C	Formula[b]	λ_{max}^{EtOH}, mμ (ε)
38	OH	CH_3	H	88.4	DMF–EtOH–H_2O	243 dec	$C_{11}H_{10}N_4O_4$	365 (3,300) 272 (12,700)
39	OH	C_2H_5	H	86.8	DMF–EtOH–H_2O	265 dec	$C_{12}H_{12}N_4O_4$	365 (3,500) 268 (15,900)
40	OH	C_2H_5	CH_3	70.9	DMF–EtOH–H_2O	232 dec	$C_{14}H_{16}N_4O_4$	367 (3,300) 272 (17,000)
41	OH	*n*-C_3H_7	CH_3	84.8	Dioxane	236 dec	$C_{15}H_{18}N_4O_4$	366 (4,000) 270 (17,600)
42	OH	*n*-C_4H_9	CH_3	73.5	DMF–EtOH	229 dec	$C_{16}H_{20}N_4O_4$	366 (4,000) 272 (17,600)
43	NH_2	CH_3	H	81.4	DMF–H_2O	266.5–269	$C_{11}H_{12}N_6O_2$	350 sh (7,000) 330 (7,300) 271 (21,700)
44	NH_2	C_2H_5	H	82.9	Dioxane–H_2O	250–252	$C_{12}H_{14}N_6O_2$	350 (sh) (6,800) 330 (7,000) 271 (22,200)
45	NH_2	$C_6H_5CH_2$	H	63.9	DMF–EtOH	261–263	$C_{17}H_{16}N_6O_2$	333 (5,000) 273 (21,600)
46	NH_2	C_2H_5	CH_3	76.0	Dioxane–H_2O	216–218	$C_{11}H_{18}N_6O_2$	332 (7,400) 272 (24,000)
47	NH_2	*n*-C_3H_7	CH_3	92.6	DMF–EtOH–H_2O	200–202	$C_{13}H_{20}N_6O_2$	333 (7,900) 273 (23,900)
48	NH_2	*n*-C_4H_9	CH_3	70.9	DMF–EtOH	214.5–216.5	$C_{16}H_{22}N_6O_2$	333 (8,200) 273 (24,200)
49	NH_2	$C_6H_5CH_2CH_2$	CH_3	88.9	Dioxane–H_2O	275.5–276	$C_{20}H_{22}N_6O_2$	333 (8,300) 272 (24,000)

[a] Recrystallized product. [b] Satisfactory analysis was obtained for C, H, and N.

(a pH 5 with concentrated HCl and placed in the cold for 2 days. The crude solid was isolated by filtration and dried (P_2O_5). After recrystallization from the appropriate solvent, the purified material was dried (P_2O_5) at 100° *in vacuo* overnight.

6-(*N*-Alkyl-*N*-arylamino)uracils (27–32, Table III).—A mixture of the *N*-alkyl-*N*-arylamine (0.02 mol) and 6-chlorouracil (0.01 mol) was heated, as described above, at 195–200°. The crude product was isolated by procedures described for the 2-amino-4-chloro-6-(*N*-alkyl-*N*-arylamino)pyrimidines.

2,4-Diamino-6-(*N*-alkyl-*N*-arylamino)pyrimidine·HCl (33–37, Table III).—A mixture of the *N*-alkyl-*N*-arylamine (0.02 mol) and 6-chloro-2,4-diaminopyrimidine (0.02 mol) was allowed to react and the crude product isolated as described for the preparation of the 2-amino-4-chloro-6-(*N*-alkyl-*N*-arylamino)pyrimidines. After the aqueous ammoniacal mixture was kept in the cold overnight, the aqueous layer was decanted, the residual oil or semisolid was dissolved in hot C_6H_6 (100–150 ml) and dried (Na_2SO_4), and excess dry HCl was introduced into the C_6H_6 solution. After standing in the cold overnight, the crude salt was isolated by filtration.

6-(*N*-Alkyl-*N*-arylamino)-5-nitrouracils (38–42, Table IV). To a filtered solution of 6-chloro-5-nitrouracil[17] (0.02 mol) in EtOH (200 ml) was added a solution of the *N*-alkyl-*N*-arylamine (0.02 mol) and Et_3N (0.02 mol) in EtOH (50 ml). After refluxing ($CaCl_2$ tube) for 0.5 hr, 150 ml of the EtOH was removed by distillation. The mixture was cooled in an ice bath and the yellow crystals were separated by filtration, washed with cold EtOH, and dried.

2,4-Diamino-6-(*N*-alkyl-*N*-arylamino)-5-nitropyrimidines (43 49, Table IV).—A solution of 6-chloro-2,4-diamino-5-nitropyrimidine[12] (0.020 mol) and the *N*-alkyl-*N*-arylamine (0.022 mol) was refluxed in DMF (30 ml) for 15 min. After cooling, the mixture was poured with vigorous stirring into dilute NH_4OH (150 ml). The crude product was collected by filtration, washed with H_2O, air dried, and crystallized.

2,4-Diamino-6-(*N*-ethyl-3,4-dimethylanilino)-5-nitrosopyrimidine Hydrochloride (50).—To a solution of **34** (2.95 g, 0.01 mol), 1.0 *N* HCl (10 ml), and H_2O (70 ml) was added, at 5°, dropwise over 10 min, a solution of $NaNO_2$ (0.690 g, 0.01 mol) in H_2O (10 ml). Stirring was continued in the cold for 1 hr. After standing in the cold overnight, the red solid was collected by filtration and recrystallized twice from H_2O to give 1.58 g (48.9%) of the analytically pure product which darkened at 230° and decomposed at *ca.* 240°: λ_{max}^{EtOH} (ε) 500 sh mμ (10,300), 482 (11,400), 378 11,800), 340 sh (6300), 279 (38,100). *Anal.* ($C_{14}H_{19}ClN_6O$) C, H, Cl, N.

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