The Synthesis and Biological Activity of Substituted 2,6-Diaminopyridines[1]

Diether G. Markees,

Department of Chemistry and Physics, Wells College, Aurora, New York

Virginia C. Dewey, and George W. Kidder

Biological Laboratory, Amherst College, Amherst, Massachusetts

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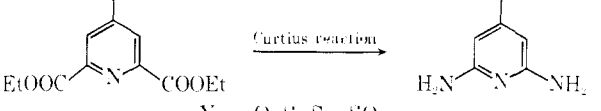
A series of 4-alkoxy-, 4-aralkoxy-, and 4-aryloxy-2,6-diaminopyridines, and some of their thio and seleno analogs, was prepared by Curtius reactions with appropriately substituted dialkyl dipicolinates. The amines were tested for growth inhibition of *Tetrahymena pyriformis* W. and *Crithidia fasciculata*. For significant inhibitory activity the substituent must be an alkoxy group containing at least four carbon atoms, or, preferably, a phenoxy group. If the hydrocarbon group is linked to the pyridine moiety by sulfur or selenium, the inhibitory activity of the diamine against *T. pyriformis* W. is reversed by Tween 80. The chemical property common to the most strongly reversed diamines seems to be the inability of the substituent to serve as a base in hydrogen bonding.

Some time ago we reported the synthesis and some of the biological properties of a series of 4-alkoxy-2,6-diaminopyridines.[2] This paper deals with a continuation of that investigation. To explore more fully the structural requirements for antiprotozoal activity of this type of compounds we prepared 2,6-diaminopyridines with various substituents in position 4. Previous results showed that increased length of the alkoxy group was paralleled by increased growth inhibition of *Tetrahymena pyriformis* W. and *Crithidia fasciculata* and that the most active compound of the series was 4-*n*-hexyloxy-2,6-diaminopyridine. Since bulk or lipophilic character of the substituent in position 4 was implicated among the factors in modifying the activity of these compounds, representatives varying in these parameters were prepared. Also included in this series are compounds in which the ether oxygen has been replaced by other atoms or a sulfonyl group.

As in the previous work, esters of appropriately substituted pyridine-2,6-dicarboxylic acids were subjected to Curtius reactions to give the desired diamines.



Y = O, S, Se, SO_2

R = CH_2=$CHCH_2$, $CH_3OCH_2CH_2$, C_6H_5, $C_6H_5(CH_2)_n$ (n = 1, 2, 3), p-ClC_6H_4, C_6H_5CH=$CHCH_2$, C_3H_5, n-C_4H_9

The required esters with substituents containing sulfur or selenium were prepared according to published procedures.[3] The remaining starting materials were obtained by reaction of the sodium compound of diethyl 4-hydroxypyridine-2,6-dicarboxylate with a suitable alkyl halide. We found that this reaction leads to ethers, in contrast to the alkylation of the sodium salts of various 2- and 4-hydroxypyridines which yields N-alkylpyridones.[4] The synthesis of diethyl 4-ethoxypyridine-2,6-dicarboxylate from diethyl 4-chloropyridine-2,6-dicarboxylate and sodium ethoxide[5] and comparison with the material obtained by reaction of sodium diethyl 4-hydroxypyridine-2,6-dicarboxylate and ethyl iodide provided the necessary evidence for the course of the reaction. Further evidence was obtained by comparison of the ultraviolet spectra of N- and O-ethylchelidamic acid and the acids obtained by saponification of the reaction product of sodium diethyl 4-hydroxypyridine-2,6-dicarboxylate and phenethyl bromide and by reaction of chelidamic acid with phenethylamine. Diethyl 4-phenoxypyridine-2,6-dicarboxylate was prepared by reaction of diethyl chelidamate with diphenyliodonium chloride.[6] Saponification of the ester gave the corresponding acid which could be decarboxylated to 4-phenoxypyridine.

The Curtius reactions were carried out as usual, except that in several cases the reaction of the intermediate hydrazides with nitrous acid was done in aqueous DMF. No efforts were made to purify the intermediate azides. The crude products were converted to the carbamates which were hydrolyzed to the amines. 4-(3-Phenylpropoxy)-2,6-dicarbethoxyamidopyridine was obtained by hydrogenation of 4-cinnamyloxy-2,6-dicarbethoxyamidopyridine.

The organisms used and the culture methods for the biological tests were essentially the same as the ones reported previously.[2] All compounds, as well as representatives of the series published earlier, were tested on the ciliate both in presence and in absence of Tween 80 (polyoxyethylene sorbitan monooleate). In experiments with *Crithidia*, Tween 80 was replaced by Triton WR 1339 (*p*-isooctylpolyoxyethylenephenol polymer) at 0.5 mg/ml. Folic acid at 2 μg/ml was replaced by a mixture of biopterin and folic acid each at 0.001 μg/ml. Stocks were maintained in a medium depleted of pteridines.

The effect of the various compounds on the growth of *T. pyriformis* in absence and in presence of Tween 80 and on *C. fasciculata* are summarized in Table I. 4-Phenoxy-2,6-diaminopyridine proved to be about four times as effective an inhibitor of *Tetrahymena* as 4-*n*-hexyloxy-2,6-diaminopyridine in the absence of Tween 80 and about 18 times as effective in its presence.

(1) Presented (in part) before the Division of Medicinal Chemistry, at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 1962. This investigation was supported in part by Grants AM1005 and CA0294 from the National Institutes of Health, U. S. Public Health Service, and by Grants G-14387 and GB-770 from the National Science Foundation.

(2) D. G. Markees, V. C. Dewey, and G. W. Kidder, *Arch. Biochem. Biophys.*, **86**, 179 (1960).

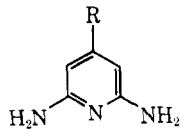
(3) D. G. Markees, *J. Org. Chem.*, **28**, 2530 (1963).

(4) See, *e.g.*, H. S. Mosher in "Heterocyclic Compounds," Vol. 1, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950, p 535.

(5) D. G. Markees and G. W. Kidder, *J. Am. Chem. Soc.*, **78**, 4130 (1956).

(6) Method suggested by Dr. John D. Roberts, California Institute of Technology.

TABLE I
INHIBITION OF GROWTH OF PROTOZOA BY 4-SUBSTITUTED 2,6-DIAMINOPYRIDINES



	Concn causing half max inhib, μmoles/ml			
		T. pyriformis		
R	*C. fasciculata*	Tween 80 absent	Tween 80 added	Ratio
CH_3O[a]	...	1.06	2.26	2.1
n-C_5H_9O[a]	0.199	0.098	0.243	2.5
i-$C_5H_{11}O$[a]	0.200	0.078	0.491	6.3
n-$C_5H_{11}O$[a]	0.125	0.035	0.228	6.5
n-$C_6H_{13}O$[a]	0.069	0.035	0.324	9.2
$C_6H_5CH_2O$	0.241	0.0480	0.332	6.6
$C_6H_5CH_2CH_2O$	0.17	0.0382	0.365	9.6
$C_6H_5(CH_2)_3O$	0.0528	0.0136	0.164	12.8
CH_2=$CHCH_2O$	0.648	0.454	0.512	1.1
C_6H_5CH=$CHCH_2O$	0.48	0.0474	0.614	13.0
$CH_3OCH_2CH_2O$	2.30	2.17	1.05	0.46
C_6H_5O	0.0283	0.0084	0.0179	2.4
C_6H_5S	0.086	0.0236	0.364	18.6
C_6H_5Se	0.0333	0.0067	0.217	32.5
C_2H_5S	0.615	0.232	0.414	1.78
n-C_4H_9S	0.19	0.0436	0.431	9.9
$C_6H_5CH_2S$	0.102	0.0330	0.770	23.5
p-ClC_6H_4S	0.0734	0.0105	0.4	38
$C_2H_5SO_2$	2.5	...	...	...[b]
n-$C_4H_9SO_2$	2.18	...	...	...[b]
$C_6H_5SO_2$	c	...	...	...

[a] See ref 2. [b] No inhibition at 200 μg/ml. [c] No inhibition at solubility limit.

Only 4-phenylseleno-2,6-diaminopyridine was more active, at least in the absence of Tween 80. 4-Phenoxy-2,6-diaminopyridine was also the most effective inhibitor of *Crithidia*.

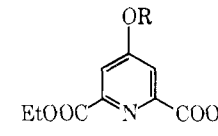
In general, the inhibitory effects are parallel for the two organisms. Where the order varies, the difference between the effects is small, as in the case of 4-phenoxy-2,6-diaminopyridine and 4-phenylseleno-2,6-diaminopyridine or of 4-cinnamyloxy- and 4-benzyloxy-2,6-diaminopyridine. The inhibition of *T. pyriformis* by all compounds except 4-(2-methoxyethoxy)-2,6-diaminopyridine is partially reversed by Tween 80. This reversal, however, is not caused by oleic acid, which could conceivably be available to the organism from the Tween, since neither the acid nor its alkali salts show any effect on inhibition.

As shown earlier,[2] 4-alkoxy-2,6-diaminopyridines interfere with the metabolism or function of biopterin in *C. fasciculata* and with lipid metabolism in *T. pyriformis*. *Crithidia* has been found to require biopterin for the metabolism of various lipid compounds[7] and for the hydroxylation of aromatic rings.[8] Therefore it would appear that these inhibitors are competing with unconjugated pteridines for an enzyme or enzymes concerned with the metabolism of lipids or aromatic compounds. Such enzymes would in all probability have active sites of a hydrophobic nature to facilitate substrate binding. The observation that increasing hydrophobic character of the substituent on the diaminopyridine is associated with increased inhibitory activity might be explained on this basis. The effectiveness of Tween 80 in reversing inhibition seems to parallel the number of carbon atoms in the alkoxy, aralkoxy, or alkylthio derivatives of diaminopyridine. This effect may be connected with the relative hydrophobic nature of the substituent under consideration. Such a view is supported by the strong reversal by Tween 80 of inhibition exerted by most of the thioethers (and 4-phenylseleno-2,6-diaminopyridine) since the S or Se atom would not serve as a site for hydrogen bonding with water. Furthermore, the inhibition by 4-(2-methoxyethoxy)-2,6-diaminopyridine which has an additional oxygen available for hydrogen bonding is actually increased by addition of Tween 80.

The comparison of the activities of 4-phenoxy-, 4-*n*-hexyloxy-, and 4-phenylthio-2,6-diaminopyridine in the presence and absence of the wetting agent suggests that factors other than molecular size or the ability to participate in H bonds influence the effectiveness of the inhibitors. In particular, the inhibitors having a phenyl substituent (*e.g.*, 4-phenoxy-2,6-diaminopyridine) might have a special affinity for the enzyme(s) carrying out hydroxylations of aromatic compounds.

That the nature of the group linking the hydrocarbon moiety to the pyridine ring is important in determining the inhibitory activity is further shown by replacement of the ether or thioether bridge by a sulfonyl group which leads to compounds devoid of significant activity in our tests.

TABLE II
DIETHYL ESTERS OF SUBSTITUTED 2,6-PYRIDINEDICARBOXYLIC ACIDS



R	Yield, %	Mp, °C	Recrystn solvent	Formula	Anal.
CH=$CHCH_2$	87	60–62	Hexane	$C_{14}H_{17}NO_5$	N
$CH_3OCH_2CH_2$	55	61–63	Hexane	$C_{14}H_{19}NO_6$	N
$C_6H_5CH_2$	83	79–81	Ether or petr ether	$C_{18}H_{19}NO_5$	C, H
$C_6H_5CH_2CH_2$	90	Oil[a]		$C_{19}H_{21}NO_5$	
C_6H_5CH=$CHCH_2$	52	83–85	Ether	$C_{20}H_{21}NO_5$	N
C_6H_5	46	78–78.5	Dil EtOH	$C_{17}H_{17}NO_5$	C, H

[a] Saponified to acid for identification, yield 68%, mp 181–183° (from EtOAc). *Anal.* ($C_{15}H_{13}NO_5$) C, H, N.

Experimental Section[9]

Substituted Diethyl Pyridine-2,6-dicarboxylates.—Dehydrated diethyl 4-hydroxypyridine-2,6-dicarboxylate was dissolved in absolute EtOH and mixed with an absolute EtOH solution of 1 equiv of Na. The mixture was refluxed for a short time and cooled. The precipitation of the sodium derivative was completed by addition of ether to the cooled mixture and the product was collected and dried. Reflux of this salt with an excess of the appropriate halide for 15–20 hr brought about the formation of the ether linkage. Further preparative information and analytical data are summarized in Table II. The preparation of

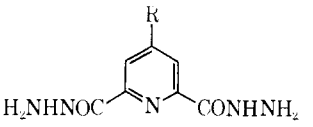
(7) (a) G. W. Kidder and V. C. Dewey, *Biochem. Biophys. Res. Commun.*, **12**, 280 (1963); (b) V. C. Dewey and G. W. Kidder, *Federation Proc.*, **23**, 376 (1964); (c) V. C. Dewey and G. W. Kidder, *Arch. Biochem. Biophys.*, **115**, 401 (1966).

(8) (a) G. W. Kidder in "Chemical Zoology," Vol. I, M. Florkin and B. Scheer, Ed., Academic Press Inc., New York, N. Y., 1967; (b) S. Kaufman in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., Pergamon Press, Oxford, England, 1964, p 307; (c) F. D. Marshal, S. K. Chatterjee, and E. M. Gal, *Federation Proc.*, **23**, 429 (1964).

(9) The melting points were taken on a Mel-Temp apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Table III

Substituted Pyridine-2,6-dicarboxylic Acid Dihydrazides

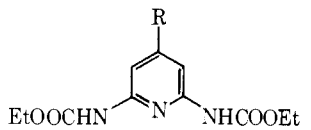


R	Yield, %	Mp, °C	Recrystn solvent	Formula	Analyses
CH_2=$CHCH_2O$	81	217–219	H_2O	$C_{10}H_{13}N_5O_3$	N
$CH_3OCH_2CH_2O$	89	219–220	EtOH	$C_{10}H_{15}N_5O_4$	N
$C_6H_5CH_2O$	79	206–208.5	MeCN	$C_{14}H_{15}N_5O_3$	N
$C_6H_5CH_2CH_2O$	53	184–185.5	H_2O	$C_{15}H_{17}N_5O_3$	N
C_6H_5CH=$CHCH_2O$	86	172–175	EtOH	$C_{16}H_{17}N_5O_3$	N
C_6H_5O	96	244–247.5	EtOH	$C_{12}H_{13}N_5O_3$	N
C_2H_5S[a]	95	211–213	Dioxane	$C_9H_{13}N_5O_2S$	N
n-C_4H_9S	95	191–193	EtOH	$C_{11}H_{17}N_5O_2S$	N
$C_6H_5CH_2S$	69	224–228	EtOH	$C_{14}H_{15}N_5O_2S$	N
C_6H_5S	91	210–212	H_2O	$C_{13}H_{13}N_5O_2S$	[b]
p-ClC_6H_4S	81	>300	DMF	$C_{13}H_{12}ClN_5O_2S$	N
C_6H_5Se	54[c]	214–215	EtOH–H_2O	$C_{13}H_{13}N_5O_2Se$	N
$C_2H_5SO_2$	98	215–217	H_2O	$C_9H_{13}N_5O_4S$	N
n-$C_4H_9SO_2$	95	192–193	H_2O	$C_{11}H_{17}N_5O_4S$	N
$C_6H_5SO_2$[d]	50	256	H_2O	$C_{13}H_{13}N_5O_4S\cdot 2H_2O$	C, H, N

[a] Prepared from methyl ester in MeOH solution. [b] *Anal.* N: calcd, 23.1; found, 23.6. [c] With respect to diethyl 4-chloropyridine-2,6-dicarboxylate; diethyl 4-phenylselenopyridine-2,6-dicarboxylate did not crystallize and was not further characterized. [d] Prepared in dioxane solution.

Table IV

Substituted 2,6-Dicarbethoxyamidopyridines



R	Yield, %	Mp, °C	Recrystn solvent	Reaction medium	Formula	Analyses
CH_2=$CHCH_2O$	57	102–103	EtOH–H_2O	1.5 *M* HCl	$C_{14}H_{19}N_4O_6$	N
$CH_3OCH_2CH_2O$	22	108	EtOH–H_2O	1 *M* HCl	$C_{14}H_{21}N_3O_6$	N
$C_6H_5CH_2O$	45	118–120	EtOH–H_2O	1 *M* HCl[a]	$C_{18}H_{21}N_3O_6$	N
$C_6H_5(CH_2)_2O$	40	104–105.5	EtOH	3 *M* HCl–DMF (1:4)	$C_{19}H_{23}N_3O_6$	N
$C_6H_5(CH_2)_3O$[b]	78	114–115	EtOH	..	$C_{20}H_{25}N_3O_6$	C, H, N
C_6H_5CH=$CHCH_2O$	34	140–141	EtOH	3 *M* HCl–DMF (1:4)	$C_{20}H_{23}N_3O_6$	N
C_6H_5O	42	133–135.5	EtOH–H_2O	3 *M* HCl–DMF (1:10)	$C_{17}H_{19}N_3O_6$	N
C_2H_5S	56	114–117	EtOH–H_2O	3 *M* HCl–DMF (1:4)	$C_{13}H_{19}N_3O_4S$	N
C_4H_9S	18	136–137	EtOH	3 *M* HCl–DMF (2:5)	$C_{15}H_{23}N_3O_4S$	N
$C_6H_5CH_2S$	41	136–137	EtOH	[c]	$C_{18}H_{21}N_3O_4S$	N
C_6H_5S	25	161.5–163	C_6H_6	AcOH–H_2O (1:1)	$C_{17}H_{19}N_3O_4S$	N
p-ClC_6H_5S	57	174–175	EtOH	12 *M* HCl–DMF (1:11)	$C_{17}H_{18}ClN_3O_4S$	N
C_6H_5Se	47	149–152	EtOH	3 *M* HCl–DMF (1:4)	$C_{17}H_{19}N_3O_4Se$	N
$C_2H_5SO_2$	59	125–127	EtOH–H_2O	1.5 *M* HCl	$C_{13}H_{19}N_3O_6S$	N
$C_6H_5SO_2$	48	174–175	EtOAc	1.5 *M* HCl	$C_{17}H_{17}N_3O_6S$	N

[a] The gummy crude product was extracted (C_6H_6) and worked up. [b] Prepared by low pressure hydrogenation of 4-cinnamyloxy-2,6-dicarbethoxyamidopyridine at room temperature in dioxane over Pd–C. [c] 1 *M* HCl–AcOH–DMF (3:3:2), 6 moles of HCl used.

diethyl 4-ethoxypyridine-2,6-dicarboxylate may serve as illustration of the procedure. A mixture of 5.0 g of sodium diethyl 4-hydroxypyridine-2,6-dicarboxylate and 40 ml of EtI was refluxed for 17 hr. Volatile material was removed, water was added to the residue, and the product was taken up with ether. The solvent was removed after washing (Na_2CO_3, H_2O) and the crude ester (3.9 g, 76%) was collected, mp 87–87.5° (from petroleum ether) undepressed by admixture of authentic material. *Anal.* ($C_{13}H_{17}NO_5$) C, H, N.

Diethyl 4-Phenoxypyridine-2,6-dicarboxylate.—Anhydrous diethyl 4-hydroxypyridine-2,6-dicarboxylate[10] (9.6 g), 12.6 g of diphenyl iodonium chloride,[11] and 50 ml of absolute EtOH were added to a solution of 1.0 g of Na in 25 ml of the same solvent. Most of the EtOH was removed after reflux for 22 hr and water was added to the residue. The product was extracted with three portions of ether, the combined extracts were washed (Na_2CO_3, H_2O), the solution was dried, and the ether was evaporated; the product crystallized on cooling. Further preparative and analytical information is presented in Table II.

4-Phenoxypyridine-2,6-dicarboxylic Acid.—Saponification of 5.0 g of diethyl 4-phenoxypyridine-2,6-dicarboxylate with 75 ml of 2.5 *M* NaOH followed by acidification with HCl gave 2.7 g (66%) of the acid, mp 192–194° dec, from H_2O. *Anal.* ($C_{13}H_9NO_5$) C, H, N.

Decarboxylation of this acid gave **4-phenoxypyridine**, mp 45°, bp 132° (2 mm) (lit.[12] mp 44–45°).

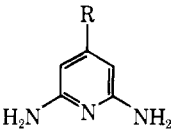
(10) D. G. Markees, *J. Org. Chem.*, **29**, 3120 (1964).

(11) (a) F. M. Beringer, M. Drexler, E. M. Gindler, and C. C. Lumpkin, *J. Am. Chem. Soc.*, **75**, 2705 (1953); (b) F. M. Beringer, E. J. Geering, I. Kuntz, and M. Mausner, *J. Phys. Chem.*, **60**, 141 (1956).

(12) D. Jerchel, H. Fischer, and K. Thomas, *Chem. Ber.*, **89**, 2921 (1956).

TABLE V

SUBSTITUTED 2,6-DIAMINOPYRIDINES



R	Yield, %	Mp, °C	Recrystn solvent	Formula	Analyses
CH_2=$CHCH_2O$	51	114.5–116	C_6H_6–hexane or H_2O[a]	$C_8H_{11}N_3O$	C, H, N
$CH_3OCH_2CH_2O$	53	97–99	C_6H_6	$C_8H_{13}N_3O_2$	C, H, N
$C_6H_5CH_2O$	67	166–168.5	EtOH–H_2O	$C_{12}H_{13}N_3O$	C, H, N
$C_6H_5CH_2CH_2O$	54	133–135	C_6H_6–hexane	$C_{13}H_{15}N_3O$	C, H, N
$C_6H_5CH_2CH_2CH_2O$	79	138	EtOH–H_2O	$C_{14}H_{17}N_3O$	C, H, N
C_6H_5CH=$CHCH_2O$		146–147	H_2O	$C_{14}H_{15}N_3O$	C, H, N
C_6H_5O	86	195	EtOH–H_2O	$C_{11}H_{11}N_3O$	C, H, N
C_2H_5S	85	165	EtOH	$C_7H_{11}N_3S$	C, H, N, S
n-C_4H_9S	87	157–159	EtOH–H_2O	$C_9H_{15}N_3S$	C, H, N, S
$C_6H_5CH_2S$	95	202–203	EtOH	$C_{12}H_{13}N_3S$	C, H, N, S
C_6H_5S	78	108–110	C_6H_6[a]	$C_{11}H_{11}N_3S$	C, H, N, S
p-ClC_6H_5S	70	133–135	C_6H_6	$C_{11}H_{10}ClN_3S$	C, H, N, Cl, S
C_6H_5Se	33	99–100	EtOH	$C_{11}H_{11}N_3Se$	C, H, N
$C_2H_5SO_2$	63	170–171.5	EtOH	$C_7H_{11}N_3O_2S$	C, H, N, S
n-$C_4H_9SO_2$	32	161–163	H_2O	$C_9H_{15}N_3O_2S$	C, H, N, S
$C_6H_5SO_2$	47	285 dec[b]	n-PrOH	$C_{11}H_{11}N_3O_2S$	C, H, N, S

[a] Also sublimed. [b] After darkening.

N-Phenethylpyridone-2,6-dicarboxylic Acid.—A mixture of 20 ml of phenethylamine, 5.0 g of chelidonic acid, and 70 ml of EtOH was refluxed for 20 hr. The crystals filtered from the hot mixture were dissolved in H_2O (100 ml) and the solution was acidified. The colorless precipitate (1.5 g 20%) was recrystallized from aqueous EtOH, mp 175° dec. *Anal.* ($C_{15}H_{13}NO_5$) C, H, N.

Substituted Pyridine-2,6-dicarboxylic Acid Dihydrazides.—Excess hydrazine hydrate was added to the solution of the requisite ester in a five- to tenfold amount of EtOH. The mixture was refluxed for several hours and cooled, and the precipitate was collected. Additional preparative and analytical information is contained in Table III.

Substituted 2,6-Dicarbethoxyamidopyridines.—To a solution of the appropriate hydrazide in the reaction medium which contained 3–6 equiv of HCl was added with stirring a solution of $NaNO_2$ in a mixture of H_2O and DMF which would not materially change the solvent composition of the reaction medium. The temperature was kept at 10–15°. The azide usually precipitated as a powdery solid and was filtered, washed (H_2O), air dried, and suspended in an adequate amount of absolute EtOH. This mixture was refluxed until the evolution of N_2 ceased. Part of the alcohol was distilled and the carbamate which had separated was collected. If no solid formed on cooling the EtOH solution, water was added to the mixture to precipitate the product. More preparative information and analytical data are contained in Table IV.

Substituted 2,6-Diaminopyridines.—An alcoholic solution of the proper carbamate was added to a solution of the same weight of KOH in a 20-fold amount of 95% EtOH and the mixture was refluxed for several hours. If a crystalline precipitate formed on cooling it was filtered and decomposed by acidification and warming, and the free amine was precipitated by addition of dilute NaOH. If the potassium carbamate failed to crystallize, most of the alcohol was removed, H_2O was added to the residue, and the resulting mixture was worked up as above. Additional preparative information and analytical data are contained in Table V.