Studies on Anticoccidial Agents. 12. Synthesis and Anticoccidial Activity of Methyl-2(6)-nitro- and -3(5)-nitropyridinecarboxamides[1]

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A number of methyl-2- and -3-nitropyridinecarboxamides have been synthesized. It has been established that the presence of at least one hydrogen atom, adjacent to the NO_2 function, is important for anticoccidial activity and introduction of a methyl group to the adjacent position of the $CONH_2$ function sometimes confers enhanced activity. Among the compounds herein, 5- and 6-methyl-2-nitropyridine-4-carboxamides possess optimal anticoccidial activity, being as potent as the parent compound.

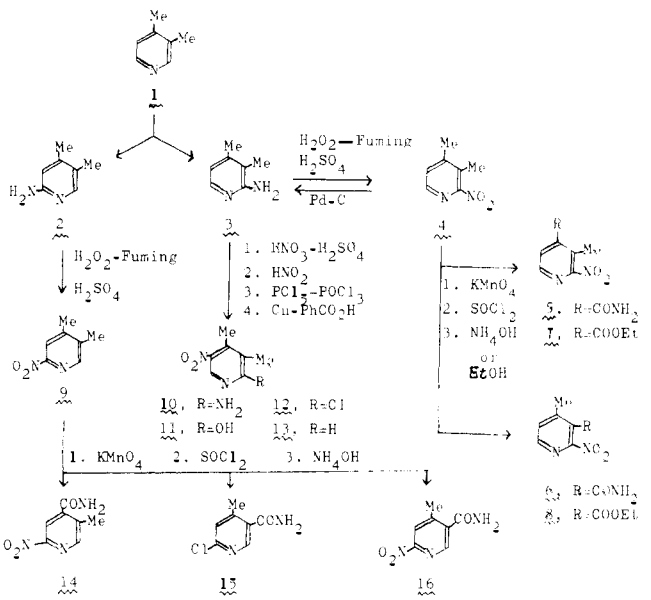
In previous papers[2] we reported that the 2-, 3-, 5-, and 6-nitropyridinecarboxamides, but not the 4-nitro analogue, possess anticoccidial activity against *Eimeria tenella*, and also among methyl-5-nitronicotinamides, the 2- and 4-methyl-, but not the 6-methyl-, analogues are significantly effective, showing that the activity is greatly affected by the position of the nitro group and the methyl group. These observations prompted us to extend our synthetic program to elucidate the effect of the methyl moiety in nitropyridinecarboxamides on coccidiostatic activity.

Chemistry. (A) Methyl-2(6)-nitropyridinecarboxamides. Starting from 2,3-, 2,4-, 2,5-, 3,4-, and 3,5-dimethylpyridines, a variety of methyl-2-nitropyridinecarboxamides **5**, **6**, **14**, **16**, **25**, **26**, **36**, **40**, **41**, **46**, and **47** were prepared as shown in Schemes I–V and Table I. 3,4-Dimethylpyridine (**1**) was aminated[3] and oxidized with H_2O_2 and fuming H_2SO_4 to give isomeric nitro compounds, **4** and **9**. The structure of 3,4-dimethyl-2-nitropyridine (**4**) was confirmed by catalytic reduction to the known 2-amino-3,4-dimethylpyridine hydrochloride[3] (**3**) while the structure of the other isomer, **9**, was determined by the fact that **9** differed from 3,4-dimethyl-5-nitropyridine (**13**), obtainable from **3** by nitration followed by diazotization, hydrolysis, chlorination, and dechlorination. Oxidation of dimethylnitropyridine **4** with $KMnO_4$, followed by chlorination with $SOCl_2$ and ammonolysis, gave the isomeric amides **5** and **6**, whereas ethanolysis of the acid chlorides gave the ethyl esters **7** and **8**. The ester **7** was easily converted to the amide **5** by ammonolysis; however, under the same reaction conditions the isomer **8** was recovered unchanged, possibly because of the steric hindrance of the nitro and the methyl group, thus explaining the individual structures of **5**–**8**. To confirm its structure, 4-methyl-2-nitronicotinamide (**6**) was prepared by an unequivocal route from 2,6-dichloro-4-methylnicotinonitrile (**17**)[4] through 2-chloro-4-methylnicotinonitrile (**18**) as shown in Scheme II. A similar synthetic approach was conducted for the preparation of 5-methyl-2-nitroisonicotinamide (**14**) and 4-methyl-6-nitronicotinamide (**16**) (Scheme I). The structure of **14** was established by x-ray analysis and that of **16** was identified by preparation from **20** through 6-chloro-4-methylnicotinamide (**15**) as shown in Scheme II.

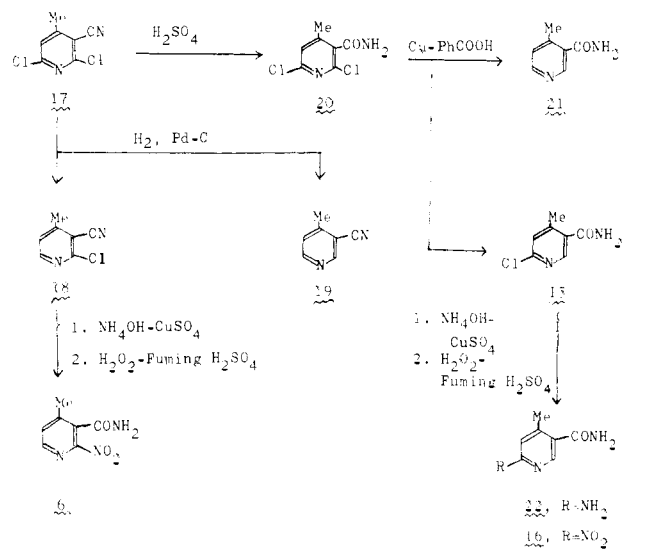
4-Methyl-6-nitropyridine-2-carboxamide (**25**) and 6-methyl-2-nitroisonicotinamide (**26**) were analogously prepared from 2-amino-4,6-dimethylpyridine (**23**)[5] via the nitro compound **24**. For confirmation of the structures of these isomeric compounds, **25** and **26**, the latter was prepared by another synthetic method; amination of 2-chloro-6-methylisonicotinic acid (**27**)[6] with NH_4OH and $CuSO_4$, followed by oxidation with H_2O_2 and fuming H_2SO_4, produced the nitro acid **28**. Treatment of the acid **28** with $SOCl_2$ and then with NH_4OH afforded the amide **26**.

For the synthesis of 5-methyl-6-nitronicotinamide (**36**), 3,5-dimethyl-2-nitropyridine (**30**) was prepared as usual

Scheme I



Scheme II



by oxidation of 2-amino-3,5-dimethylpyridine (**29**)[3] with H_2O_2 and fuming H_2SO_4. Oxidation of **30**, followed by chlorination and methanolysis, resulted in the formation of the isomeric chloro esters **31** and **32**, in which the relative position of the substituents was assigned by nuclear Overhauser effect (NOE) measurements; The 100-MHz NMR spectrum of **31** had aromatic proton doublets at δ 8.13 for C_4-H and 8.81 for C_2-H (J = 2.3 Hz), while the isomer **32** had values of δ 7.95 for C_4-H and 8.31 for C_6-H (J = 2.5 Hz). Irradiation of the ring methyl group

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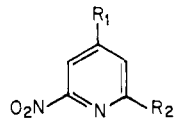
Table I. Physical Properties of 2-Nitropyridine Derivatives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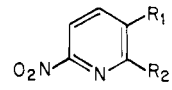
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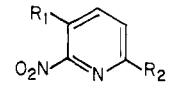
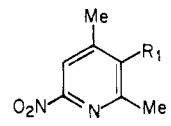
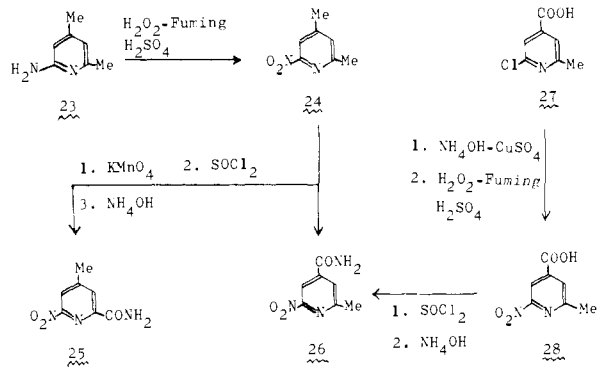

type III


type IV

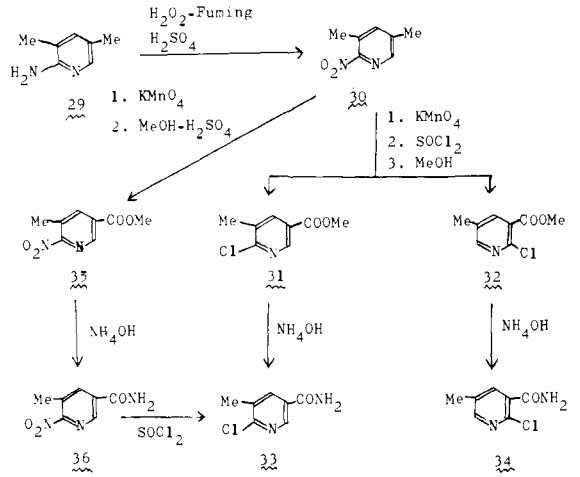
No.	Type	R_1	R_2	Method[a]	Yield, %[b]	Mp, °C	Formula[c]
24	I	Me	Me	A	21.9	68-69[d]	$C_7H_8N_2O_2$
25	I	Me	$CONH_2$	B	9.7	184-185[e]	$C_7H_7N_3O_3$
26	I	$CONH_2$	Me	B	3.6	227-228[f]	$C_7H_7N_3O_3$
37	II	Me	Me	A	44.5	88-89[f]	$C_7H_8N_2O_2$
38	II	COOMe	Me	I	13.0	72-73[g]	$C_8H_8N_2O_4$
39	II	Me	COOMe	I	13.0	94-95[g]	$C_8H_8N_2O_4$
40	II	$CONH_2$	Me	D	74.0	190-191[h]	$C_7H_7N_3O_3$
41	II	Me	$CONH_2$	D	91.0	167-168[f]	$C_7H_7N_3O_3$
43	III	Me	Me	A	28.5	Oil	$C_7H_8N_2O_2$
44	III	COOMe	Me	I	11.1	51-52[i]	$C_8H_8N_2O_4$
45	III	Me	COOMe	I	22.0	71-72[i]	$C_8H_8N_2O_4$[j]
46	III	$CONH_2$	Me	D	56.9	225-227[i]	$C_7H_7N_3O_3$
47	III	Me	$CONH_2$	D	43.4	165-166[i]	$C_7H_7N_3O_3$
50	IV	CN		A	15.4	52-53[f]	$C_8H_7N_3O_2$
51	IV	$CONH_2$		F	60.6	179-180[f]	$C_8H_9N_3O_3$

[a] The letter refers to the general method given in the Experimental Section. [b] The yield of the analytically pure compounds isolated is given. [c] Satisfactory analyses have been obtained for C, H, and N. [d] From aqueous EtOH. [e] From acetone-petroleum ether. [f] From EtOAc-*n*-hexane. [g] From EtOAc. [h] From EtOH. [i] From EtOAc-petroleum ether. [j] Anal. Calcd for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.60; H, 4.15; N, 13.83.

Scheme III

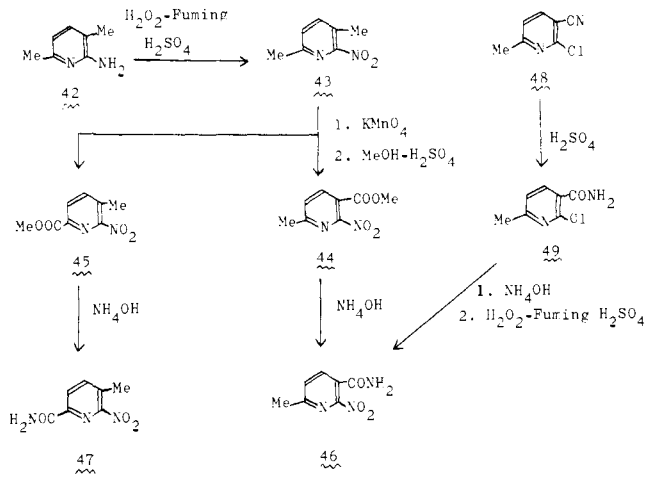


Scheme IV



at δ 2.44 in **31** resulted in a 14% enhancement of the C_4-H signal and irradiation of the methyl group at δ 2.36 in **32** also resulted in 10 and 6% enhancements of the C_4-H and C_6-H, respectively. These results indicate that **31** has one proton adjacent to the methyl group and **32** has two protons adjacent to this group. Aqueous ammonia converted these chloro esters, **31** and **32**, into the amides, **33** and **34**. In this route, unexpectedly the nitro group was

Scheme V

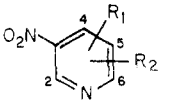


replaced by the chlorine atom. Therefore, the intermediate acid was directly esterized with MeOH and H_2SO_4 to give the nitro ester **35** as the only isolable product. Ammonolysis of **35** produced the amide **36**, whose structure was established by conversion to the chloroamide **33** with $SOCl_2$. Furthermore, structural assignment was reconfirmed by NMR spectral examination of **35**; a 13% NOE enhancement in the C_4-H (δ 8.40, J = 2.0 Hz) and no area enhancement in the C_2-H (δ 8.94, J = 2.0 Hz) were observed when the methyl group at δ 2.56 was irradiated.

2-Methyl-6-nitronicotinamide (**40**) and 3-methyl-6-nitropyridine-2-carboxamide (**41**) shown in Table I were prepared from 2-amino-5,6-dimethylpyridine[7] via the isomeric esters **38** and **39**, whose structures were determined by NOE. Irradiation of the methyl group at δ 2.93 in **38** had no effect on the doublets at δ 8.09 (J = 8.2 Hz) and 8.49 (J = 8.2 Hz) of the pyridyl protons, whereas irradiation of the methyl group at δ 2.72 in **39** resulted in a 16% enhancement of the C_4-H signal of the pyridyl protons (C_4-H at δ 7.96, J = 8.2 Hz, and C_5-H at δ 8.27, J = 8.2 Hz).

Similar transformations were also effected in the series of 6-methyl-2-nitronicotinamide (**46**) and 3-methyl-2-

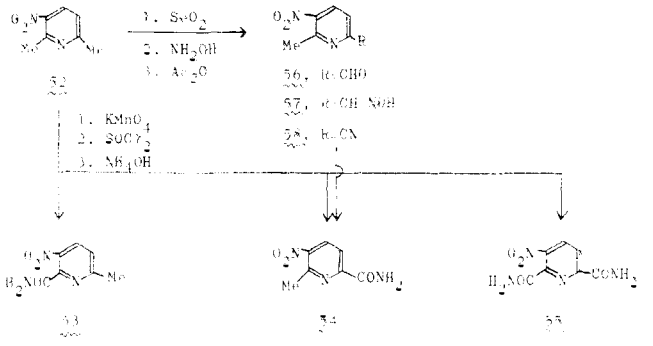
Table II. Physical Properties of 3-Nitropyridine Derivatives



No.	R_1	R_2	Method[a]	Yield, %[b]	Mp, °C	Formula[c]
53	2-$CONH_2$	6-Me	B	4.1	160-162[d]	$C_7H_7N_3O_3$
54	2-Me	6-$CONH_2$	B	25.8	170-171[d]	$C_7H_7N_3O_3$
55	2-$CONH_2$	6-$CONH_2$	B	6.1	264-266[e]	$C_7H_6N_4O_4$
59	2-$CONH_2$	5-Me	B	23.4	212-213[f]	$C_7H_7N_3O_3$
60	2-Me	5-$CONH_2$	B	28.0	181-182[g]	$C_7H_7N_3O_3$
61	4-$CONH_2$	5-Me	B	31.6	213-215[f]	$C_7H_7N_3O_3$
62	2-$CONH_2$	4-Me	H	30.2	243-244[g]	$C_7H_7N_3O_3$
63	2-Me	4-$CONH_2$	H	52.1	203-204[h]	$C_7H_7N_3O_3$
64	4-Me	6-$CONH_2$	H	24.0	180-182[f]	$C_7H_7N_3O_3$
65	2,4-Me_2	6-COOMe	I	24.5	108-109[f]	$C_9H_{10}N_2O_4$
66	2,4-Me_2	6-$CONH_2$	D	64.5	145-147[g]	$C_8H_9N_3O_3$
67	5-Me	6-$CONH_2$	F	71.4	152-153[f]	$C_7H_7N_3O_3$
68	5-Me	6-Br	J	37.9	57-58[f]	$C_6H_5BrN_2O_2$
69	5-Me	6-CN	K	53.3	75-76[f]	$C_7H_5N_3O_2$

[a-c] See corresponding footnotes in Table I. [d] From EtOAc. [e] From DMF-EtOAc. [f] From EtOAc-*n*-hexane. [g] From EtOH. [h] From EtOH-petroleum ether.

Scheme VI



nitropyridine-6-carboxamide (**47**) starting from 2-amino-3,6-dimethylpyridine (**42**)[3] as shown in Scheme V. For elucidation of the individual structures, the amide **46** was prepared from 2-chloro-6-methylnicotinonitrile (**48**).[8]

2,4-Dimethyl-6-nitronicotinamide (**51**) was readily obtained from 6-amino-2,4-dimethylnicotinonitrile[9] by oxidation with H_2O_2 and fuming H_2SO_4 and partial hydrolysis with H_2SO_4.

(B) Methyl-3(5)-nitropyridinecarboxamides. Starting from 2,4-, 2,5-, 2,6-, 4,5-, and 4,6-dimethylpyridines, methyl-3(5)-nitropyridinecarboxamides **53**, **54**, **59–63**, and **64** were prepared (Table II). Earlier reports[10,11] described that oxidation of 2,6-dimethyl-3-nitropyridine **52** with $KMnO_4$ gave 2-methyl-3-nitropyridine-6-carboxylic acid and/or 3-nitropyridine-2,6-dicarboxylic acid. The present work showed that $KMnO_4$ oxidation of **52** produced three acids, which were converted to two monoamides **53** and **54** and a diamide **55** by chlorination with $SOCl_2$ and ammonolysis. In order to ascertain the individual structures, 2-methyl-3-nitropyridine-6-carboxamide (**54**)[11] was prepared from 6-formyl-2-methyl-3-nitropyridine (**56**)[12] by oximation, dehydration with Ac_2O, and partial hydrolysis with H_2SO_4 (Scheme VI).

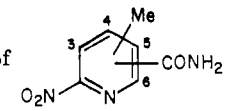
It has been reported[13] that 2,5-dimethyl-3-nitropyridine was converted to 5-methyl-3-nitropyridine-2-carboxylic acid in two steps by oxidation with SeO_2 and HNO_3. $KMnO_4$ oxidation of this dimethylnitropyridine gave two acids, which could be transformed to amides **59** and **60** in 23.4 and 28.0% yields, respectively, with $SOCl_2$ and NH_4OH. The former, **59**, was identical with the sample prepared from the known 5-methyl-3-nitropyridine-2-carboxylic acid by the same method mentioned above. Similar transformations have been effected in 5-methyl-3-nitroisonicotinamide (**61**) starting from 4,5-dimethyl-3-nitropyridine (**13**). The structural identification was based on physical data and the fact that compound **61** is not identical with another isomer, 4-methyl-5-nitronicotinamide.[2a]

The amides **62–64** were prepared from the corresponding acids[10] by treatment with $SOCl_2$ and NH_4OH, the amide **66** was prepared from the acid[14] by esterification and ammonolysis, and 5-methyl-3-nitropyridine-6-carboxamide (**67**) was obtained from 6-hydroxy-5-methyl-3-nitropyridine[15] by bromination with PBr_3, followed by cyanation[16] with CuCN and hydrolysis.

Biological Results. The target compounds were tested against *E. tenella* using a 1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride (Amprolium) resistant strain according to the previously described procedure[17] and the results, shown in Tables III and IV, were compared with those of the parent nitropyridinecarboxamides. For an ACI above 180, the anticoccidial activity was determined as excellent, 180–160 as marked, 160–140 as moderate, 140–120 as slight, and below 120 as inactive.

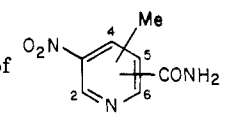
2-Nitropyridine-4-carboxamide[2b] possesses the highest order of activity among the nitropyridinecarboxamides. Introduction of the 5- or 6-methyl group (**14** and **26**) showed almost the same order of activity, but introduction of the 3-methyl group (**5**) gave a compound of diminished activity. In the 2-nitropyridine-5-carboxamide derivatives, 4-methyl, 6-methyl, and 4,6-dimethyl compounds (**16**, **40**, and **51**) were much more effective than the parent compound, but the isomeric 3-methyl derivative **36** was less active. In the analogues of 2-nitropyridine-6-carboxamide, the structure–activity relationships follow a similar pattern to those observed in the former two series. Introduction of the 5-methyl group (**41**) adjacent to $CONH_2$ enhanced the activity, but introduction of the 3-methyl group (**47**) adjacent to the NO_2 group reduced the activity. The activity of 2-nitropyridine-3-carboxamide was slightly enhanced by introduction of the 4-methyl group (**6**). Thus, appended methyl groups provided position-dependent effects, which might be caused by either electronic and/or steric interactions.

3-Nitropyridinecarboxamides were also evaluated. For analogues belonging to 3-nitropyridine-4-carboxamide, introduction of the 2-methyl group (**63**) adjacent to the NO_2 group reduced activity, but the 5-methyl substitution (**61**), adjacent to the $CONH_2$ group, contributed greatly

Table III. Anticoccidial Activity of 

No.	Position of Me	Position of $CONH_2$	Concn of drug in feed, %	ACI[a]
2-Nitropyridine-4-carboxamide			0.015	195
			0.007	195
5	3	4	0.015	177
			0.007	128
14	5	4	0.015	197
			0.007	186
26	6	4	0.015	196
			0.007	193
2-Nitropyridine-5-carboxamide			0.007	122
36	3	5	0.015	131
			0.007	89
16	4	5	0.015	190
			0.007	172
40	6	5	0.015	195
			0.007	173
51	4,6	5	0.015	180
			0.007	158
2-Nitropyridine-6-carboxamide			0.015	164
47	3	6	0.015	118
25	4	6	0.015	172
41	5	6	0.015	181
2-Nitropyridine-3-carboxamide			0.015	135
6	4	3	0.015	150
46	6	3	0.015	120

[a] ACI = percent survival + percent relative weight gain − lesion score − oocyst score.

Table IV. Anticoccidial Activity of 

No.	Position of Me	Position of $CONH_2$	Concn of drug in feed, %	ACI[a]
3-Nitropyridine-4-carboxamide			0.015	165
63	2	4	0.015	149
61	5	4	0.015	198
			0.007	162
3-Nitropyridine-2-carboxamide			0.015	132
62	4	2	0.015	115
59	5	2	0.015	128
53	6	2	0.015	139
3-Nitropyridine-6-carboxamide			0.015	165
54	2	6	0.015	164
64	4	6	0.015	182
67	5	6	0.015	180
66	2,4	6	0.015	97

[a] See footnote *a* in Table III.

to the activity. In 3-nitropyridine-2-carboxamide, introduction of the 5- or 6-methyl group (**59** and **53**) resulted in only small changes in its properties, but 4-methyl substitution (**62**), adjacent to the NO_2 moiety, led to reduction in activity. In the case of 3-nitropyridine-6-carboxamide, addition of the 5-methyl group (**67**) at the ortho position of $CONH_2$ and the 4-methyl group (**64**) contributed to the activity and the 2-methyl group (**54**) at the ortho position of NO_2 resulted in only small changes in the activity, but the 2,4-dimethyl group (**66**), which occupied both ortho positions of NO_2, led to reduction of the activity. From these observations, it can be concluded that the presence of at least one hydrogen atom adjacent to the NO_2 function is important for the activity and enhanced anticoccidial activity is sometimes conferred by introduction of the methyl group to the adjacent position of the $CONH_2$ function. Among the compounds herein, 5- and 6-methyl-2-nitropyridine-4-carboxamides possess optimal anticoccidial activity, being as potent as the parent compound.[2a]

Experimental Section

Melting points are uncorrected. IR and NMR were determined on a Perkin-Elmer 221 and a Varian A-60 and HA-100, respectively. Spectral data were consistent with the assigned structures. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.3% of the theoretical values. Typical experimental procedures are described.

3,4-Dimethyl-2-nitropyridine (4) and 3,4-Dimethyl-6-nitropyridine (9). Following the procedure of Albert et al.,[3] 3,4-dimethylpyridine (1, 192 g, 1.79 mol) was aminated at 165–170 °C under N_2 for 8 h with *N,N*-dimethylaniline (280 mL, 2.21 mol) and $NaNH_2$ (80 g, 2.05 mol) to give a crystalline mixture of 2-aminodimethylpyridines **2** and **3** (90.0 g, 41.1%).

Method A. A solution of the crude aminodimethylpyridines **2** and **3** (15 g, 0.12 mol) in concentrated H_2SO_4 (60 mL) was added dropwise below 20 °C to a mixture of fuming H_2SO_4 (250 mL) and 30% H_2O_2 (125 mL). The mixture was stirred at 15–18 °C for 15 h, poured into ice–water, neutralized with Na_2CO_3 and extracted with Et_2O. The extract was dried and the solvent was removed to give a yellow crystalline residue, which was chromatographed over silica gel, and each product isolated was recrystallized from EtOAc–*n*-hexane to afford **4** (5.6 g, 30.0%), mp 68–69 °C, and **9** (1.7 g, 9.1%), mp 96–97 °C. Anal. (both compounds, $C_7H_8N_2O_2$) C, H, N.

2-Amino-3,4-dimethylpyridine (3). A solution of **4** (1.52 g, 10 mmol) in MeOH (50 mL) was hydrogenated over 10% Pd/C (1.5 g). After separation of the catalyst, the solvent was removed to leave a crystalline product, which was recrystallized from EtOAc–*n*-hexane to afford **3** (1.0 g, 80.3%), mp 81–82 °C. The amine hydrochloride had mp 240 °C (lit.[3] mp 239–240 °C). Anal. ($C_7H_{10}N_2$) C, H, N.

3-Methyl-2-nitroisonicotinamide (5) and 4-Methyl-2-nitronicotinamide (6). Method B. To a solution of 3,4-dimethyl-2-nitropyridine (**4**, 19 g, 0.125 mol) in H_2O (1300 mL) containing Na_2CO_3 (1.2 g) was added $KMnO_4$ (60 g, 0.38 mol) portionwise at 80 °C with vigorous stirring. Stirring and heating were continued for 1 h, and after separation of MnO_2, the reaction mixture was shaken with benzene. The organic layer was washed with H_2O and dried and the solvent was removed to give the starting material recovered (2.1 g, 11.1%). The aqueous layer was concentrated into a small volume and acidified to pH 1.5 with HCl, and the solution was concentrated to dryness to give a crystalline residue. The crude acids containing inorganic matter thus obtained were treated with $SOCl_2$ (300 mL) under reflux for 3 h. After separation of the insoluble matter, excess reagent was removed and the acid chloride (11.2 g) was stirred with 28% NH_4OH (110 mL) at room temperature for 1 h, neutralized with HCl, and extracted with EtOAc. The extract was dried and the solvent was removed to leave a crystalline residue, which was chromatographed over silica gel eluting with EtOAc–benzene. The first eluate yielded **5** (3.2 g, 14.16%), mp 127–128 °C. The second eluate gave **6** (0.52 g, 2.3%), mp 189 °C. Anal. (both compounds, $C_7H_7N_3O_3$) C, H, N.

Ethyl 3-Methyl-2-nitroisonicotinate (7) and Ethyl 4-Methyl-2-nitronicotinate (8). Method C. 3,4-Dimethyl-2-nitropyridine (**4**, 4.5 g, 30 mmol) was oxidized with $KMnO_4$ and then chlorinated with $SOCl_2$ as described above. The starting material recovered was 0.8 g. The acid chlorides obtained were added portionwise to cooled EtOH (15 mL) and the mixture was stirred at room temperature for 1 h, diluted with H_2O, neutralized with $NaHCO_3$, and extracted with EtOAc. The extract was dried and the solvent was removed to give an oil (4.1 g), which was chromatographed over silica gel to give **7** (1.2 g, 19.3%) as an oil and **8** (2.1 g, 33.8%) as an oil. Anal. (both compounds, $C_9H_{10}N_2O_4$) C, H, N.

Ammonolysis of Ethyl 3-Methyl-2-nitroisonicotinate (7) and Ethyl 4-Methyl-2-nitronicotinate (8). Method D. (A) A suspension of **7** (0.5 g, 2.4 mmol) in 28% NH_4OH (15 mL) was stirred at room temperature for 24 h. The mixture was neutralized with dilute HCl under cooling and extracted with EtOAc. The extract was dried and the solvent was removed to leave an oil, which was crystallized by addition of *n*-hexane. Recrystallization from EtOAc–*n*-hexane gave **5** (0.23 g, 53.5%), mp 127–128 °C. Anal. ($C_7H_7N_3O_3$) C, H, N.

(B) A suspension of 8 (0.5 g, 2.4 mmol) in 28% NH_4OH (15 mL) was worked up as described above to give the starting material recovered (0.48 g, 96.0%).

2-Chloro-4-methylnicotinonitrile (18). A mixture of 2,6-dichloro-4-methylnicotinonitrile[4] (**17**, 22 g, 0.118 mol), AcONa (18 g), and $PdCl_2$ (0.25 g) in MeOH (100 mL) was shaken in a H_2 atmosphere (50 psi) for 1 h. After removal of the catalyst, the solution was concentrated to a small volume, diluted with H_2O, neutralized with $NaHCO_3$, and extracted with EtOAc. The extract was dried, the solvent was removed, and the residue obtained was chromatographed over silica gel eluting with EtOAc–*n*-hexane (1:1). The first eluate gave 18 (1.3 g, 7.2%), mp 105–108 °C, on recrystallization from EtOAc: NMR (DMF-d_7) δ 2.63 (s, 3 H, C-4 methyl), 7.64 (1, H, d, J = 5.0 Hz), 8.62 (1, H, d, J = 5.0 Hz). Anal. ($C_7H_5ClN_2$) C, H, Cl, N. The second eluate gave 4-methylnicotinonitrile (**19**, 10.1 g, 72.3%), mp 44–45 °C (lit.[4] mp 45–46 °C). Anal. ($C_7H_6N_2$) C, H, N.

4-Methyl-2-nitronicotinamide (6). A suspension of 18 (1.2 g, 7.9 mmol) in 28% NH_4OH (5 mL) containing $CuSO_4{\cdot}5H_2O$ (0.12 g, 0.48 mmol) was heated at 170 °C for 20 h in a sealed tube. The cooled reaction mixture was bubbled with H_2S, the precipitated CuS was separated, and the filtrate was concentrated to dryness to give a crystalline residue (1.1 g), which was dissolved in concentrated H_2SO_4 (3.5 mL). This solution was added dropwise to a mixture of 30% fuming H_2SO_4 (19 mL) and 30% H_2O_2 (9 mL), and the mixture was stirred at room temperature for 48 h, poured into ice water, neutralized with Na_2CO_3, and extracted with EtOAc. The extract was dried and the solvent was removed to leave a crystalline residue, which was recrystallized from EtOAc–petroleum ether to give a pure product **6** (0.2 g, 14%), mp 189 °C. Anal. ($C_7H_7N_3O_3$) C, H, N. IR and NMR spectra were identical with the sample **6** obtained from **4**.

2-Amino-3,4-dimethyl-5-nitropyridine (10). 2-Amino-3,4-dimethylpyridine (**3**, 7.4 g, 60 mmol) was added portionwise under cooling to concentrated H_2SO_4 (60 g), and to this solution concentrated HNO_3 (4.6 mL, d 1.42) was added dropwise below 10 °C. The mixture was stirred at room temperature for 16 h and then the temperature was raised gradually to 95 °C. After stirring was continued at 95 °C for 2 h, the mixture was poured into ice water and neutralized with NH_4OH to give a crystalline product, which was recrystallized from EtOAc to yield **10** (3.9 g, 38.5%), mp 240 °C. Anal. ($C_7H_9N_3O_2$) C, H, N.

2-Hydroxy-3,4-dimethyl-5-nitropyridine (11). An aqueous solution (2 mL) of $NaNO_2$ (1.6 g, 23 mmol) was added dropwise below 10 °C to a solution of **10** (2.5 g, 15 mmol) in 2.5 NH_2SO_4 (40 mL), and the mixture was stirred for 30 min. The crystals which deposited were collected and recrystallized from EtOAc to afford an analytically pure sample of 11 (2.2 g, 87.7%), mp 220–222 °C. Anal. ($C_7H_8N_2O_3$) C, H, N.

2-Chloro-3,4-dimethyl-5-nitropyridine (12). A mixture of 11 (2.0 g, 12 mmol) in PCl_5 (3.3 g) and $POCl_3$ (2 mL) was heated at 110–120 °C for 8 h and then poured to ice water, neutralized with $NaHCO_3$, and extracted with EtOAc. The extract was dried and the solvent was removed to give **12** (1.8 g, 81.5%) as an oil.

3,4-Dimethyl-5-nitropyridine (13). Method E. To a stirred mixture of **12** (7.0 g, 38 mmol) and benzoic acid (14 g, 114.8 mmol) at 150–160 °C was added portionwise Cu powder (10 g). After addition was completed, stirring and heating were continued for 10 min. The mixture was cooled and shaken with aqueous Na_2CO_3 solution and $CHCl_3$. The $CHCl_3$ extract was washed with water and dried, and the solvent was removed to give an oil, which was purified by silica gel chromatography to afford **13** (1.5 g, 26.3%), mp 42–43 °C. Anal. ($C_7H_8N_2O_2$) C, H, N.

5-Methyl-2-nitroisonicotinamide (14), 6-Chloro-4-methylnicotinamide (15), and 4-Methyl-6-nitronicotinamide (16). 3,4-Dimethyl-6-nitropyridine (**9**, 6.2 g, 40 mmol) was oxidized with $KMnO_4$ and the acids obtained were treated with $SOCl_2$ and then with NH_4OH as described in method B. The starting material (0.3 g, 4.8%) was recovered. The products were separated by silica gel chromatography eluting with EtOAc–*n*-hexane. The first eluate afforded 6-chloro-4-methylnicotinamide (**15**, 0.58 g, 8.4%), mp 166–167 °C, on recrystallization from EtOH. Anal. ($C_7H_7ClN_2O$) C, H, Cl, N. The second eluate gave a mixture of **14** and **16**, which was rechromatographed over silica gel to afford two pure amides. 6-Nitro-3-methylisonicotinamide (**14**, 0.42 g, 5.7%) had mp 202–204 °C from EtOH. Anal. ($C_7H_7N_3O_3$) C, H, N. 6-Nitro-4-methylnicotinamide (**16**, 0.11 g, 1.5%) had mp 178–179 °C from EtOH. Anal. ($C_7H_7N_3O_3$) C, H, N.

2,6-Dichloro-4-methylnicotinamide (20). Method F. A solution of 2,6-dichloro-4-methylnicotinonitrile (**17**, 3.0 g, 16 mmol) in concentrated H_2SO_4 (10 mL) was stirred at 100 °C for 1 h, poured into ice water, made alkaline with $NaHCO_3$, and extracted with EtOAc. The extract was washed with water and dried, and the solvent was removed to leave a crystalline residue. Recrystallization from EtOAc–*n*-hexane gave a pale yellow crystalline product (**20**, 2.3 g, 69.7%), mp 173–175 °C. Anal. ($C_7H_6Cl_2N_2O$) C, H, Cl, N.

6-Chloro-4-methylnicotinamide (15). The dichloropyridine **20** (1.0 g, 4.9 mmol) was treated with Cu powder (1.25 g, 20 mmol) and benzoic acid (1.75 g, 15 mmol) as described in method E. The products were separated by silica gel chromatography to give three compounds. The least polar substance was the starting material **20** (0.21 g, 21.3%), mp 173–175 °C. The second eluate gave **15** (0.12 g, 14.5%), mp 169–170 °C, on recrystallization from EtOAc–*n*-hexane: NMR (DMF-d_7) δ 2.47 (3 H, s, C-4 methyl), 7.3–8.2 (2 H, br, $CONH_2$), 7.42 (1 H, s), 8.45 (1 H, s). Anal. ($C_7H_7ClN_2O$) C, H, Cl, N. The last eluate gave 4-methylnicotinamide (**21**, 0.012 g, 1.8%), mp 166–167 °C (lit.[4] mp 167–167.5 °C), on recrystallization from EtOH. Anal. ($C_7H_8N_2O$) C, H, N.

6-Amino-4-methylnicotinamide (22). Method G. A mixture of **15** (7.2 g, 42 mmol) and 28% NH_4OH (25 ml) containing $CuSO_4{\cdot}5H_2O$ (0.7 g, 2.8 mmol) was heated at 170 °C for 16 h in a sealed tube and cooled to give a crystalline product, which was separated and recrystallized from EtOH–*n*-hexane to afford **22** (4.5 g, 70.5%), mp 189–191 °C dec. Anal. ($C_7H_9N_3O$) C, H, N.

4-Methyl-6-nitronicotinamide (16). This material was prepared in 23.1% yield from **22** by method A and was recrystallized from EtOH: mp 178–180 °C. Anal. ($C_7H_7N_3O_3$) C, H, N.

6-Methyl-2-nitroisonicotinic Acid (28). A mixture of the chloro acid **27**[6] (4.0 g, 23 mmol), 28% NH_4OH (20 mL), and $CuSO_4{\cdot}5H_2O$ (0.4 g) was heated at 155 °C for 20 h in a sealed tube. To the cooled reaction mixture was added Na_2S, and precipitated CuS was separated. The filtrate was concentrated into dryness and the residue was dissolved in water and acidified to pH 4.0 with HCl to give 2-amino-6-methylisonicotinic acid (2.7 g, 76.2%), mp 250 °C. Anal. ($C_7H_8N_2O_2$) C, H, N.

The amino acid was oxidized with H_2SO_4 and H_2O_2 (method A) to nitro acid **28** in a 29.3% yield, mp 169–170 °C dec, on recrystallization from water. Anal. ($C_7H_6N_2O_4$) C, H, N.

6-Methyl-2-nitroisonicotinamide (26). Method H. The acid **28** (1.0 g, 5.5 mmol) was treated with $SOCl_2$ (20 mL) under reflux for 3 h and the excess $SOCl_2$ was removed to give an oil, which was added portionwise to cooled 28% NH_4OH (15 mL). After 1 h, the mixture was neutralized with HCl and extracted with EtOAc. The extract was dried, the solvent was removed, and the residue was recrystallized from EtOAc–*n*-hexane to give **26** (0.3 g, 30.0%), mp 227–228 °C. Anal. ($C_7H_7N_3O_3$) C, H, N.

3,5-Dimethyl-2-nitropyridine (30). This pale yellow oily compound was prepared in 64.0% yield from **29**[3] by method A. Anal. ($C_7H_8N_2O_2$) C, H, N.

Methyl 6-Chloro-5-methylnicotinate (31) and Methyl 2-Chloro-5-methylnicotinate (32). These compounds were obtained in 26.7 and 13.8% yields, respectively, from dimethylnitropyridine **30** by method C. Compound **31** had mp 73–74 °C on recrystallization from EtOAc–*n*-hexane. Compound **32** was an oily material. Anal. (both compounds, $C_8H_8ClNO_2$) C, H, Cl, N.

Methyl 5-Methyl-6-nitronicotinate (35). Method I. Dimethylnitropyridine **30** (5.0 g, 33 mmol) was oxidized with $KMnO_4$ as described in method B. After extraction of the starting material (1.5 g, 9.9%) recovered with EtOAc, the aqueous solution was

concentrated and acidified to give crystalline products. The crude acids were esterified with MeOH (150 mL) containing concentrated H_2SO_4 (2 mL) under reflux for 3 h. The reaction mixture was concentrated into dryness, diluted with water, made alkaline with Na_2CO_3, and extracted with EtOAc. The crystalline residue after evaporation of the solvent was recrystallized from EtOAc–*n*-hexane to give **35** (2.2 g, 34.4%), mp 104–106 °C. Anal. ($C_8H_8N_2O_4$) C, H, N.

6-Chloro-5-methylnicotinamide (33). (A) Treatment of **31** (0.5 g, 2.7 mmol) with 28% NH_4OH at room temperature for 16 h gave **33** (0.28 g, 60.9%), mp 211–212 °C. Anal. ($C_7H_7ClN_2O$) C, H, Cl, N.

(B) Nitronicotinamide **36** (0.2 g, 1.0 mmol) was refluxed in $SOCl_2$ (10 mL) for 3 h. The IR and NMR spectra of the compound (0.07 g, 37.2%), mp 211–212 °C, were identical with that of **33** obtained above.

2-Chloro-5-methylnicotinamide (34). This compound was prepared in 48.8% yield from the ester **32** using the procedure described above: mp 141–143 °C. Anal. ($C_7H_7ClN_2O$) C, H, Cl, N.

5-Methyl-6-nitronicotinamide (36). By a similar method described above, **36** was prepared from **35** in 74% yield, mp 196–198 °C. Anal. ($C_7H_7N_3O_3$) C, H, N.

2-Chloro-6-methylnicotinamide (49). This compound was prepared from **48** in 64.5% yield by method F: mp 176–178 °C. Anal. ($C_7H_7ClN_2O$) C, H, Cl, N.

6-Methyl-2-nitronicotinamide (46). By methods G and A **46** was prepared from **49** in 11% yield, mp 225–227 °C. Anal. ($C_7H_7N_3O_3$) C, H, N. The IR was superimposable with that of **46** obtained from **44**.

2-Methyl-3-nitropyridine-6-carboxamide (54). To a solution of NH_2OH in 90% EtOH (40 mL), prepared from NH_2OH–HCl (0.6 g, 8.5 mmol) and NaOAc (0.7 g, 8.5 mmol), was added portionwise a solution of the aldehyde **56**[12] (1.8 g, 10 mmol). The mixture was stirred at 80 °C for 30 min and cooled to give a crystalline product **57** (1.3 g, 66.4%), mp 217–219 °C. Anal. ($C_7H_7N_3O_3$) C, H, N.

A mixture of **57** (1.3 g, 7 mmol) and Ac_2O (10 mL) was refluxed for 12 h, cooled and poured into ice–water, made alkaline with Na_2CO_3, and extracted with $CHCl_3$. The brown oily residue after removal of the solvent was purified by silica gel chromatography to give a pale yellow oil, **58** (1.0 g, 85.5%). Anal. ($C_7H_5N_3O_2$) C, H, N.

Compound **58** (1.0 g, 6 mmol) was hydrolyzed with concentrated H_2SO_4 as described in method F and the product was recrystallized from EtOAc to give **54** (0.9 g, 81.1%), mp 170–171 °C. Anal. ($C_7H_7N_3O_3$) C, H, N. The IR spectrum of the compound was identical with that of **54** obtained from dimethylnitropyridine **52** by method B.

6-Bromo-5-methyl-3-nitropyridine (68). Method J. A mixture of 6-hydroxy-5-methyl-3-nitropyridine[15] (9.0 g, 58 mmol) and PBr_3 (45 mL) was heated at 130 °C for 2 h, cooled and poured into ice–water, made neutral with $NaHCO_3$, and extracted with EtOAc. The extract was dried and the solvent was removed to give a crystalline residue. Recrystallization from EtOAc–*n*-hexane gave **68** (4.8 g, 37.9%), mp 57–58 °C. Anal. ($C_6H_5BrN_2O_2$) C, H, Br, N. From the mother liquor, the starting material (2.5 g, 27.8%) was recovered.

6-Cyano-5-methyl-3-nitropyridine (69). Method K. A mixture of **68** (2.0 g, 9 mmol) and CuCN (1.8 g, 20 mmol) was heated at 160–165 °C for 3 h, cooled, and extracted with EtOAc. The extract was decolorized with carbon and concentrated into a small volume, and addition of *n*-hexane gave **69** (0.8 g, 53.3%), mp 75–76 °C. Anal. ($C_7H_5N_3O_2$) C, H, N.

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Antileukemic Activity of Ungeremine and Related Compounds. Preparation of Analogues of Ungeremine by a Practical Photochemical Reaction[1]

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A number of alkoxypyrrolophenanthridinium salts and their analogues related to the antileukemic alkaloid ungeremine were prepared by a practical photochemical cyclization. The importance of the quaternary nitrogen atom and of alkoxy groups, the planarity of a molecule, and steric considerations relative to antileukemic activity are discussed.

Although both the tetraalkoxydibenzo[*a*,*g*]quinolizinium salts **1**, such as coralyne,[2-7] and the tetraalkoxybenzo[*c*]phenanthridinium salts **2**, such as nitidine,[8-10] are alkoxyisoquinoline derivatives which possess activity against leukemias L1210 and P388, one structural difference is worthy of notice: the dibenzoquinolizinium salts **1** contain a relatively stabilized, "locked-in" quaternary nitrogen, wherein the N atom is at a bridgehead position of the ring structure. On the other hand, in the benzophenanthridinium salt series **2**, the quaternary nitrogen is created by alkylation after the ring system is formed. In aqueous solution the alkyl group on the quaternary nitrogen species

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