Potential Antitumor Agents. 9. 2-Formyl(*m*-amino)phenylpyridine Thiosemicarbazones

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Several *m*-nitro- and *m*-aminophenyl-substituted 2-formylpyridine thiosemicarbazones have been synthesized. *m*-Nitrophenyl-2-picolines were formed by coupling 2-picoline with diazotized *m*-nitroaniline. The 3-, 4-, 5-, and 6-substituted *m*-nitrophenyl-2-picolines were separated by fractional crystallization and their structures confirmed by nmr. These derivatives were then converted to *N*-oxides and allowed to react with Ac_2O to form corresponding 2-methyl acetates. Acid hydrolysis of esters produced carbinols, which were oxidized with MnO_2 to carboxaldehydes and then converted to thiosemicarbazones. *m*-Nitrophenyl-2-picolinaldehydes were converted to cyclic ethylene acetals which were subsequently reduced by catalytic hydrogenation to yield corresponding amino derivatives. 2-Formyl-4-(*m*-amino)phenylpyridine thiosemicarbazone was the most active antineoplastic agent of this series in mice bearing Sarcoma 180 ascites cells.

The reductive conversion of ribonucleotides to deoxyribonucleotides, catalyzed by the enzyme ribonucleoside-diphosphate reductase, is possibly a rate-controlling step in the biosynthesis of DNA. Thus, Elford, *et al.*,[1] have shown a direct correlation between the activity of ribonucleoside-diphosphate reductase and the growth rate of a series of hepatomas. Two other enzymes involved in DNA synthesis, thymidylate synthetase and thymidine kinase, did not demonstrate such a close degree of correlation with tumor growth rate.

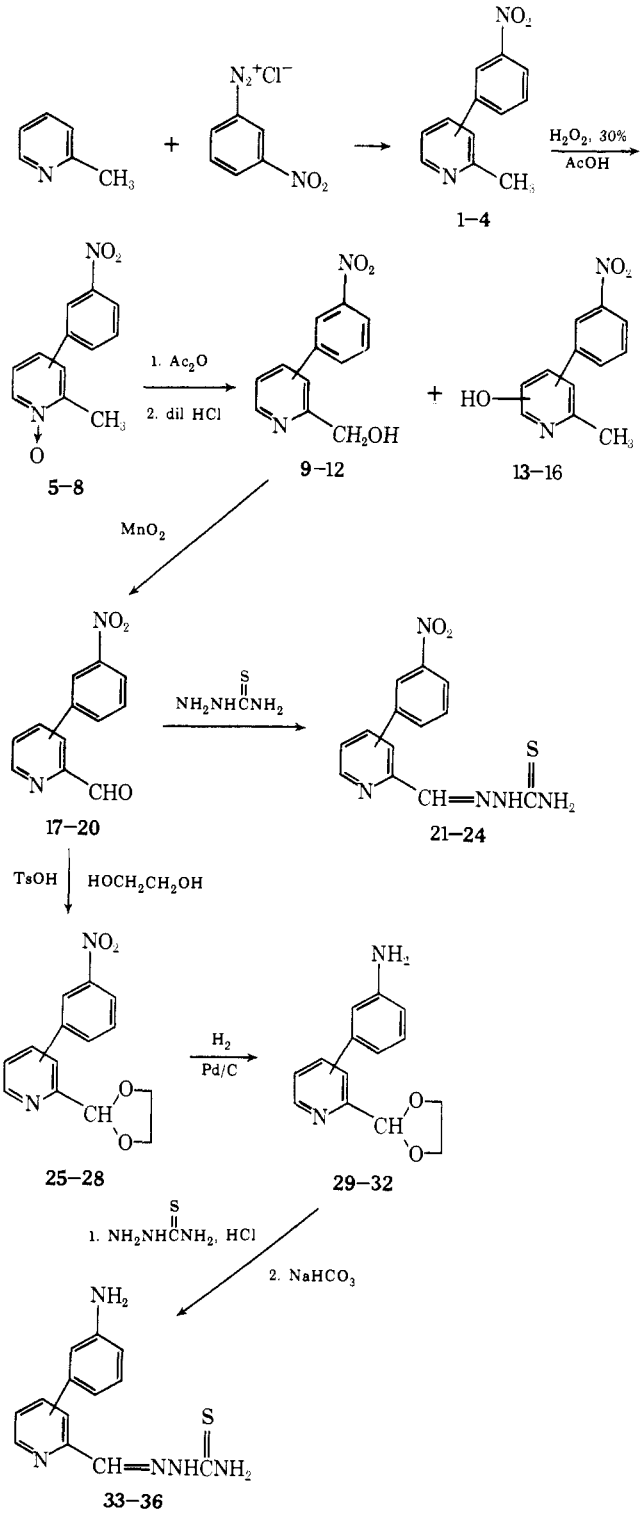
α-(N)-Heterocyclic carboxaldehyde thiosemicarbazones primarily block DNA synthesis in mammalian cells by inhibiting the enzyme ribonucleoside-diphosphate reductase.[2-6] These compounds have been shown to possess significant antineoplastic activity against a variety of transplanted animal tumors,[7-16] spontaneous lymphomas of dogs,[17] and DNA viruses of the Herpes group.[6] One of the members of this series, 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP), has been tested for antineoplastic activity in man. However, its marked tumor-inhibitory potency in animal systems was not duplicated in patients with cancer.[18,19] The relative inactivity of 5-HP appeared to be the result of (a) its relatively low inhibitory potency for the target enzyme ribonucleoside-diphosphate reductase[5] and (b) its short biological half-life in man because of the rapid formation and elimination of the glucuronide conjugate.[18]

Kinetic studies of the molecular mechanism of action of this class of compounds are consistent with a model in which these agents either bind as tridentate ligands to an iron-charged ribonucleoside-diphosphate reductase or a preformed iron chelate of the inhibitor interacts with the target enzyme.[20] Structure-activity relationships have delineated the bulk tolerance requirements for this interaction between enzyme, inhibitor, and ferrous ion. These studies suggested that position 6 of 2-formylpyridine thiosemicarbazone (PT) and position 3 of 1-formylisoquinoline thiosemicarbazone (IQ-1) are equivalent with respect to orientation of the inhibitor at the enzymatic binding site and that little or no tolerance exists for modification at this position.[20] The results also indicated that IQ-1, which can be visualized as a pyridine derivative with a benzene ring fused across the 3 and 4 positions, is about sixfold more potent as an inhibitor of the enzyme than is PT. Likewise, introduction of a CH_3 group on the pyridine ring of PT at either the 3, 4, or 5 positions resulted in derivatives that were better inhibitors of ribonucleoside-diphosphate reductase activity than PT. These findings suggest the possible existence of a hydrophobic bonding zone adjacent to the inhibitor-binding site of the enzyme. Therefore, in an effort to synthesize agents with greater affinity for the target enzyme, a hydrophobic moiety, such as phenyl, was introduced at various positions in PT and the hydrophilic NH_2 group was inserted to provide a means to solubilize for parenteral administration these otherwise extremely water-insoluble compounds. These derivatives were then tested for their potential as antineoplastic agents.

Chemistry. Substituted phenylpicolines were synthesized by a procedure similar to the coupling of pyridine with diazotized aniline[21] with few experimental modifications; although the reaction of 2-picoline with *m*-nitrophenyldiazonium hydrochloride has been reported,[21] individual isomers were not separated from the mixture and identified. Initially, *m*-nitroaniline was chosen as a reactant so that the NO_2 group could subsequently be reduced to an NH_2 function, which could then be utilized as the hydrochloride salt for solubilizing these α-(N)-heterocyclic carboxaldehyde thiosemicarbazones in water. The coupling reaction of diazotized *m*-nitroaniline with 2-picoline (Scheme I) gave a mixture consisting of four different isomers, the 3-, 4-, 5-, and 6-substituted *m*-nitrophenyl-2-picolines, compounds, **1**, **2**, **3**, and **4**, respectively. This reaction, although presumably a free-radical condensation, possessed a significant positional preference with regard to the attack of the pyridine ring by the *m*-nitrophenyldiazonium salt, as evidenced from the finding that the ratios of the four different isomers, **1**, **2**, **3**, and **4**, were 5:3:1:2, respectively. These yields were found to be consistent in different runs. No explanation is available for the relatively high yield (47%) of the 3 isomer **1** in this reaction. Chromatographic separation of these isomers using a variety of adsorbents and solvents was unsuccessful, and resolution of these isomers as their hydrochlorides or picrates could not be achieved. It was possible, however, to separate these isomers successfully by repetitive fractional crystallization from different solvents; this procedure is described in the Experimental Section. The characterization of the isomers was accomplished by nmr; details are described in that section.

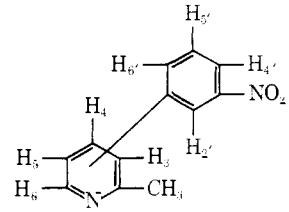
m-Nitrophenyl-2-picolines **1-4** were individually subjected to a series of reactions, as shown in Scheme I, to oxidize the 2-CH_3 group to the corresponding carboxaldehyde. This was done by first converting the *m*-nitrophenyl-2-picolines to their *N*-oxides **5-8** with 30% H_2O_2 and AcOH. The *N*-oxides were allowed to react with Ac_2O to yield mainly corresponding 2-acetoxymethyl derivatives and a small amount of a phenolic ester with an acetoxy group at some other position in the pyridine ring system. The identity of the position of this latter ester is under investigation. The separation of this mixture of esters was accomplished by hydrolysis with dilute HCl, solubilization of the phenolic compounds with alkali, and extraction of 2-hydroxymethylpyridines **9-12**. Oxidation of the

Scheme I



carbinols with MnO_2 produced the respective aldehydes 17-20 which, following reaction with thiosemicarbazide, yielded the *m*-nitrophenylpicolinaldehyde thiosemicarbazones 21-24.

In order to reduce the NO_2 group to an NH_2 function, the *m*-nitrophenylpicolinaldehydes were converted to their cyclic ethylene acetals 25-28, which were reduced by catalytic hydrogenation using Pd/C. The amino acetals 29-32 were allowed to react with thiosemicarbazide in the presence of concentrated HCl to form the desired *m*-aminophenylpicolinaldehyde thiosemicarbazones. Relevant data for compounds synthesized are listed in Table I.



1, 3-*m*-nitrophenyl
2, 4-*m*-nitrophenyl
3, 5-*m*-nitrophenyl
4, 6-*m*-nitrophenyl

Figure 1.

Nmr Studies. The nmr parameters for the compounds synthesized were consistent with the structures proposed. Differentiation of the various isomers of *m*-nitrophenyl-2-picoline 1-4 has been possible from nmr studies (Figure 1). (See paragraph at end of paper regarding supplementary material.)

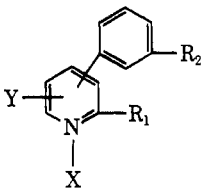
The most downfield signal of 1 at 830 Hz appeared as a quartet (J = 5, 2 Hz) and was assigned as H-6 on the basis of its chemical shift and splitting pattern. Irradiation at 830 Hz reduced the two sets of quartets at 704 (J = 8, 5 Hz) and 736 Hz (J = 8, 2 Hz) into two doublets with the same coupling constant (J = 8 Hz) indicating a simple ABX system; these were assigned to H-5 and H-4, respectively. Thus, 1 was 3-*m*-nitrophenyl-2-picoline. The 2-CH_3 signal was shifted about 15-30 Hz upfield; this differed from the other 3 isomers because it was shielded by the current effect of the phenyl ring at the 3 position, either above or below the plane of the pyridine ring, due to steric interaction of the *o*-CH_3 group. For similar reasons, the ortho protons of the phenyl ring (H-2′ and H-6′) and H-4 of the pyridine ring were slightly more shielded than in the 5 isomer 3.

The nmr spectrum of 3 contained a doublet (J = 2 Hz) at the lowest field (842 Hz), which was assigned as H-6 of the pyridine ring. The small J value of the doublet indicated α,γ coupling rather than α,β coupling. Irradiation at 842 Hz reduced the quartet at 758 Hz (J = 7.5 and 2 Hz) to a doublet (J = 7.5 Hz) and the half-width of the doublet at 704 decreased from 2.1 to 1.8 Hz; these were assigned to H-4 and H-3, respectively. Compound 3, therefore, was 5-*m*-nitrophenyl-2-picoline.

The most downfield signal of 4 at 856 Hz was a triplet (J = 1.5 Hz). Irradiation of resonance signals at 856 Hz reduced the multiplet structure around 800 Hz to two sets of quartets, one at 806 Hz (J = 7.5 and 1 Hz) and the other at 795 Hz (J = 8.5 and 1 Hz). Irradiation of the multiplets caused the triplet at 856 Hz to merge into a singlet. From the splitting pattern and the chemical shift values, the 2 protons around 800 Hz were assigned as H-4′ and H-6′ of the phenyl ring. Thus, the most downfield signal in this spectrum was due to H-2′ of the phenyl ring. The lack of a signal for the α proton of the pyridine ring indicated that compound 4 was 6-*m*-nitrophenyl-2-picoline.

Following the assignment of the 3, 5, and 6 isomers, it was clear that compound 2 was 4-*m*-nitrophenyl-2-picoline. The nmr spectrum of this isomer had much smaller $\Delta\nu$ values for the aromatic protons, due to the deshielding effects of the phenyl and pyridine rings against each other; therefore, irradiation studies could not be successfully employed. The 2-CH_3 protons of 1, 3, and 4 appeared as a sharp singlet around 250 Hz, whereas in 2, the 2-CH_3 signal was a doublet (J = 0.5 Hz). This small coupling constant apparently resulted from the long-range

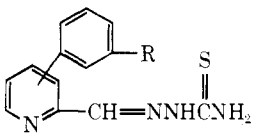
Table I



Compd	R_1	Position of phenyl substitution	R_2	X	Y	Mp, °C	Recrystn solvent	Yield, %	Formula	Analyses
1	CH_3	3	NO_2			113–113.5	Me_2CO–cyclohexane	47[a]	$C_{12}H_{10}N_2O_2$	C, H, N
2	CH_3	4	NO_2			155–156	EtOH	24[a]	$C_{12}H_{10}N_2O_2$	C, H, N
3	CH_3	5	NO_2			106	Cyclohexane	8[a]	$C_{12}H_{10}N_2O_2$	C, H, N
4	CH_3	6	NO_2			65	Pet. ether	16[a]	$C_{12}H_{10}N_2O_2$	C, H, N
5	CH_3	3	NO_2	O		187–188.5	Me_2CO–cyclohexane	95	$C_{12}H_{10}N_2O_3$	C, H, N
6	CH_3	4	NO_2	O		228–230	EtOH	88	$C_{12}H_{10}N_2O_3$	C, H, N
7	CH_3	5	NO_2	O		205–206	$CHCl_3$–Me_2CO	81	$C_{12}H_{10}N_2O_3$	C, H, N
8	CH_3	6	NO_2	O		137–138	Me_2CO–cyclohexane	52	$C_{12}H_{10}N_2O_3$	C, H, N
9	CH_2OH	3	NO_2			149.5–150.5	Me_2CO	70[b]	$C_{12}H_{10}N_2O_3$	C, H, N
10	CH_2OH	4	NO_2			133–134	C_6H_6	58[b]	$C_{12}H_{10}N_2O_3$	C, H, N
11	CH_2OH	5	NO_2			102–104	EtOH–H_2O	70[b]	$C_{12}H_{10}N_2O_3$	C, H, N
12	CH_2OH	6	NO_2			96–98	Me_2CO–cyclohexane	50[b]	$C_{12}H_{10}N_2O_3$	C, H, N
13	CH_3	3	NO_2		OH	242–243 dec	EtOH–H_2O	16	$C_{12}H_{10}N_2O_3$	C, H, N
14	CH_3	4	NO_2		OH	247–249 dec	EtOH–H_2O	7	$C_{12}H_{10}N_2O_3$	C, H, N
15	CH_3	5	NO_2		OH	187–190 dec	EtOH–H_2O	7	$C_{12}H_{10}N_2O_3$	C, H, N
16	CH_3	6	NO_2		OH	188–190 dec	EtOH–H_2O	5	$C_{12}H_{10}N_2O_3$	C, H, N
17	CHO	3	NO_2			168.5–169.5	Me_2CO	89	$C_{12}H_8N_2O_3$	C, H, N
18	CHO	4	NO_2			166–167	C_6H_6	66	$C_{12}H_8N_2O_3$	C, H, N
19	CHO	5	NO_2			167–169	$CHCl_3$–cyclohexane	77	$C_{12}H_8N_2O_3$	C, H, N
20	CHO	6	NO_2			165–166	C_6H_6	88	$C_{12}H_8N_2O_3$	C, H, N
21	$CHNNHCSNH_2$	3	NO_2			233–234 dec		92	$C_{13}H_{11}N_5O_2S$	C, H, N
22	$CHNNHCSNH_2$	4	NO_2			222–223 dec		88	$C_{13}H_{11}N_5O_2S$	C, H, N
23	$CHNNHCSNH_2$	5	NO_2			222–223 dec		95	$C_{13}H_{11}N_5O_2S$	C, H, N
24	$CHNNHCSNH_2$	6	NO_2			218–219 dec		90	$C_{13}H_{11}N_5O_2S$	C, H, N
25		3	NO_2			Oil		75	$C_{14}H_{12}N_2O_4$	
26		4	NO_2			115–116	EtOH–H_2O	90	$C_{14}H_{12}N_2O_4$	C, H, N
27		5	NO_2			98–99	C_6H_6–cyclohexane	67	$C_{14}H_{12}N_2O_4$	C, H, N
28		6	NO_2			84.5–85.5	EtOH	88	$C_{14}H_{12}N_2O_4$	C, H, N
29		3	NH_2			158–159	Me_2CO–cyclohexane	57	$C_{14}H_{14}N_2O_2$	C, H, N
30		4	NH_2			68–70	Ethyl ether	90	$C_{14}H_{14}N_2O_2$	
31		5	NH_2			145–146	Me_2CO–cyclohexane	95	$C_{14}H_{14}N_2O_2$	C, H, N
32		6	NH_2			Oil		66	$C_{14}H_{14}N_2O_2$	
33	$CHNNHCSNH_2$	3	NH_2			190–191 dec	EtOH	90	$C_{13}H_{13}N_5S$	C, H, N, S
34	$CHNNHCSNH_2$	4	NH_2			189–190 dec	EtOH–H_2O	80	$C_{13}H_{13}N_5S$	C, H, N, S
35	$CHNNHCSNH_2$	5	NH_2			203–204 dec	EtOH	84	$C_{13}H_{13}N_5S$	C, H, N, S
36	$CHNNHCSNH_2$	6	NH_2			190–192	EtOH–H_2O	91	$C_{13}H_{13}N_5S$	C, H, N, S

[a] Yields are based on the recovery of various isomers from a mixture obtained in 18% yield from the coupling reaction of *m*-nitroaniline with 2-picoline. [b] Yield based on *N*-oxide.

Table II. Effect of Substituted 2-Formylpyridine Thiosemicarbazones on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells



Compd	Position of substitution	R	Max effective daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival time, days ± S.E.	50-Day survivors[c]	% T/C[d]
Control				+18.2	13.7 ± 0.6	0/35	100
5-HP	5-OH		60	−3.3	31.4 ± 2.0	3/15	229
21	3	NO_2	10	+14.1	14.2 ± 0.7	0/5	104
22	4	NO_2	10	+15.4	15.8 ± 0.2	0/5	115
23	5	NO_2	10	+9.0	15.8 ± 1.2	0/5	115
24	6	NO_2	5	+13.9	15.8 ± 1.3	0/5	115
33	3	NH_2	5	+12.3	18.4 ± 1.9	0/5	134
34	4	NH_2	40	−7.2	32.5 ± 2.4	3/15	237
35	5	NH_2	20	+13.6	21.5 ± 3.5	1/10	157
36	6	NH_2	20	+19.9	13.6 ± 1.2	0/5	99

[a]Administered once daily for six consecutive days, beginning 24 hr after tumor implantation; dose levels were administered in a range of 5–60 mg/kg for each compound. [b]Average weight change from onset to termination of drug treatment. [c]The number of tumor-bearing animals that survived at least 50 days; these mice were calculated as 50-day survivors in the determinations of average survival time. [d]% T/C = treated/control × 100.

coupling with H-3 of the pyridine ring; such coupling was not observed with the 5 and 6 isomers.

Biological Results and Discussion. The tumor-inhibitory properties of the substituted 2-formylpyridine thiosemicarbazones were determined by measuring their effects on the survival time of mice bearing Sarcoma 180 ascites cells; the results are shown in Table II. The prolongation of life produced by the maximum effective daily dose of each compound is listed; however, a wide range of dose levels, from 5 to 60 mg/kg, was tested for each agent. For comparison, the effect of 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP), one of the most potent agents of this series, which has been tested clinically, was also measured as a positive control, and the results are included in Table II. Administration of 3-, 4-, 5-, or 6-*m*-nitrophenyl-substituted derivatives at their maximum effective daily doses did not cause impressive increases in the life span of tumor-bearing mice. However, the NH_2-substituted derivatives were all active with the exception of the 6 isomer **36**. The inactivity of **36** was consistent with our previous postulation[20] that the target enzyme, ribonucleoside-diphosphate reductase, has low bulk tolerance for the 6 position of the pyridine ring. The 4 isomer **34** was the most active agent, increasing the average survival time of tumor bearing mice from 13.7 days for untreated controls to 32.5 days. Thus, **34** was equal in activity in this system to the most active agents of this series, 5-HP and IQ-1. While IQ-1 is a water-insoluble compound, **34** can be readily solubilized as its HCl salt for parenteral administration in man. Compound **34** also has an advantage over 5-HP, in that it is not susceptible to *O*-glucuronide formation, a reaction which appeared to play a major role in the failure of 5-HP to alter significantly the course of malignant disease in man.[18] Furthermore, these compounds have been evaluated as inhibitors of ribonucleoside-diphosphate reductase;† **34** was 30 times more potent as an inhibitor of this enzyme from a rat tumor than was 5-HP. Thus, 4-(*m*-amino)phenyl-2-formylpyridine thiosemicarbazone appears to possess all of the requisite properties for consideration as a second generation drug of the α-(N)-heterocyclic carboxaldehyde thiosemicarbazone class for clinical trial.

† K. C. Agrawal, B. A. Booth, E. C. Moore, and A. C. Sartorelli, unpublished results.

Experimental Section

Melting points, determined in capillary tubes using a Thomas-Hoover stirred-liquid apparatus, are corrected. The ir absorption spectra were obtained with a Perkin-Elmer Model 257 spectrophotometer with thin films of liquids and KBr pellets of solids. Uv spectra were obtained using a Perkin-Elmer Model 402 ultraviolet–visible spectrophotometer with solutions made in absolute ethanol. Nmr spectra were determined with a JEOL 4H-100 (100 mHz) spectrometer with TMS as an internal standard. Elemental analyses were performed by the Baron Consulting Co., Orange, Conn. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values.

Antitumor Activity. Experiments were performed on CD-1 mice. Transplantation of Sarcoma 180 ascites cells was carried out as previously described using a donor mouse bearing a 7-day tumor growth.[10] Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to these agents was based on the prolongation of survival time afforded by the drug treatment.

***m*-Nitrophenyl-2-picolines (1–4).** A solution of 55.2 g (0.414 mol) of *m*-nitroaniline and 250 ml of 10% HCl was produced by heating, and to this was added 100 ml of concentrated HCl. After rapid cooling to 0°, a solution of 28.8 g (0.417 mol) of $NaNO_2$ and 70 ml of H_2O was introduced with stirring through a capillary tube beneath the surface of the solution, maintaining the reaction temperature below 0°. After complete addition, the reaction mixture was filtered (Celite), keeping the clear filtrate cold. The filtrate was added dropwise to 200 ml (2.0 mol) of 2-picoline with stirring. The reaction temperature was kept at 40°. When the addition was complete, the reaction mixture was heated at 80° for 30 min, cooled, and made basic (pH 9.0). The excess 2-picoline was flash evaporated; the residue was dissolved in 500 ml of $CHCl_3$ and filtered (Celite), washing the $CHCl_3$ with H_2O. After flash evaporation, 400 ml of 10% HCl was added and the mixture heated at 100° with stirring for 1 hr. After cooling to about 50°, the solution was filtered (Celite), the filtrate extracted with 200 ml of $CHCl_3$, and the pH adjusted to 3 (Na_2CO_3), filtered (Celite), extracted with $CHCl_3$ (3 × 150 ml) to remove organic impurities, and then made basic. The free base was extracted three times with Et_2O (200 ml each) and dried ($MgSO_4$), and the Et_2O was removed under vacuum to leave a semisolid residue that was distilled, bp 150–190° (0.1 mm); this crystallized on cooling to yield 16.2 g (18%).

Separation of Isomers. A mixture of 17.5 g of four different

isomers, obtained from the above reaction, was washed with 50 ml of Et_2O to leave 10 g of residue, mainly consisting of two isomers, which was recrystallized from 125 ml of a mixture of acetone and cyclohexane (1:4). Slow crystallization at room temperature yielded 2.4 g of **2**, mp 154–155°. The filtrate was concentrated to 100 ml and left overnight for slow crystallization. Two different types of crystals were formed: (a) large pale yellow prisms that were separated mechanically with ease, yielding 5.1 g of **1**, mp 110–112°, and (b) white granules, 0.9 g of **2**, mp 152–154°. The filtrate was further concentrated to about 25 ml and a third crop was separated in a similar manner. Each isomer was recrystallized from appropriate solvents to yield pure compounds.

The ether filtrate and the filtrate from the third crop were mixed and evaporated under vacuum to leave an oil, which was then triturated with 40 ml of a 1:1 mixture of Et_2O and petroleum ether (boiling range 30–60°) and filtered to yield 3.5 g of a mixture of the compound. Recrystallizations from cyclohexane and smaller amounts of acetone (6:1) for slower crystallization at room temperature yielded 2.0 g of **1**, 0.5 g of **2**, and finally 1.4 g of **3**, mp 105–106°. HCl gas was passed into the Et_2O and petroleum ether filtrate to form the HCl salt, which was filtered, washed with acetone, and crystallized from 60 ml of ethanol and ethyl acetate (1:1) to yield 4.0 g, mp 195–200°. The HCl salt was converted to the free base and recrystallized from petroleum ether (boiling range 30–60°) to yield 2.9 g of **4**, mp 58–60°. Two more recrystallizations from the same solvent raised the melting point to 65°. The total recovery was **1**, 7.3 g (47%); **2**, 4.2 g (24%); **3**, 1.4 g (8%); and **4**, 2.9 g (16%) from 17.5 g of the mixture.

***m*-Nitrophenyl-2-picoline *N*-Oxides (5–8).** To 5.6 g (0.262 mol) of each compound **1–4** in 15 ml of glacial AcOH, 4.5 ml of 30% H_2O_2 was added and heated at 80° with stirring. After 3 hr an additional amount of 4.5 ml of 30% H_2O_2 was added and the heating continued for another 15 hr. The reaction mixture was flash evaporated *in vacuo*, and the residue was neutralized with 10% $NaHCO_3$. The precipitate was filtered, washed with a small amount of water, dried, and crystallized from the appropriate solvent.

2-Hydroxymethyl(*m*-nitro)phenylpyridines (9–12). *m*-Nitrophenyl-2-picoline *N*-oxides (4.6 g, 0.02 mol) were heated with 45 ml of Ac_2O with stirring at 120° for 2.5 hr. The excess Ac_2O was then removed under vacuum and the esters were extracted with Et_2O; a small amount of petroleum ether was then added with stirring to the point of turbidity to precipitate the impurities. The solution was filtered through Celite and solvents were removed. The resulting esters were directly hydrolyzed by heating with 50 ml of 10% HCl at 100° for 1.5 hr. The solution was then made strongly alkaline (pH 11) with NaOH solution to dissolve the phenolic compound, cooled, and filtered. The precipitate was washed with water, dried, and recrystallized. Neutralization of the alkaline filtrate to pH 7.0 precipitated a phenolic compound (**12–16**), which was filtered, washed, and crystallized from EtOH–H_2O.

***m*-Nitrophenylpyridine-2-carboxaldehydes (17–20).** To 2.3 g (0.01 mol) of the 2-carbinols **9–12** in 100 ml of $CHCl_3$ was added 8 g of activated MnO_2 (Winthrop Laboratories), which was refluxed for 2 hr. The mixture was filtered through Celite, the $CHCl_3$ removed under vacuum, and the residue recrystallized from an appropriate solvent.

***m*-Nitrophenyl-2-formylpyridine Ethylene Acetals (25–28).** To 1.14 g (0.005 mol) of each aldehyde **17–20** in 250 ml of benzene was added 0.12 g of *p*-toluenesulfonic acid and 2 ml of ethylene glycol. The mixture was refluxed for 24 hr using a Dean-Stark trap to remove the water formed during condensation. After the reaction was complete, 50 ml of water and 5 ml of 10% $NaHCO_3$ solution were added. The benzene layer was separated, dried ($MgSO_4$), and removed under vacuum to leave the residue, which was recrystallized.

***m*-Aminophenyl-2-formylpyridine Ethylene Acetals (29–32).** The NO_2 derivatives **25–28**, 0.816 g (0.003 mol), were dissolved in 75 ml of ethanol and 0.2 g of Pd/C (10%) was added. The mixture was hydrogenated at 30 psi for 0.5 hr and filtered, and ethanol was removed under vacuum to leave a residue that was directly used for the next reaction. This residue crystallized in small amounts of ether except for compound **32**.

***m*-Aminophenyl-2-formylpyridine Thiosemicarbazones (33–36).** To 0.484 g (0.002 mol) of each acetal **29–32** in 10 ml of ethanol was added 1 ml of concentrated HCl and 0.182 g (0.002 mol) of thiosemicarbazide. The mixture was refluxed for 1 hr, whereupon the hydrochloride salt of the desired compound precipitated. The precipitate was filtered, washed with ethanol and ether, and dried. The hydrochloride salt was dissolved in 10 ml of water and neutralized with Na_2CO_3 solution. The resulting precipitate was filtered and crystallized from EtOH–H_2O.

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Supplementary Material Available. Full and expanded nmr spectra for compounds **1–4** will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-74-631.

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