24.7 g. (18%) of 4,5-dichloro-1,2-dinitrobenzene[18] (IX), m.p. 107–108°. The free base from 46.4 g. (0.21 mole) of X and 24.7 g. (0.104 mole) of IX were dissolved in 400 ml. of 80% aqueous ethanol and refluxed for 46 hr. A negative test for evolved nitric oxide was obtained with starch–potassium iodide paper at this time. The reaction mixture was diluted with 400 ml. of water and after cooling, the product was filtered. Recrystallization from 50% ethanol gave 28.7 g. (79%) of XII, m.p. 114–115°.

Anal. Calcd. for $C_{12}H_{17}Cl_2N_3O_2$: C, 42.6; H, 5.1; Cl, 21.0; N, 12.4. Found: C, 42.9; H, 5.1; Cl, 21.3; N, 12.1.

7,8-Dimethyl-10-{2-[bis(2-hydroxyethyl)amino]ethyl}isoalloxazine (IV). Procedure A.—Five grams (0.017 mole) of XI was reduced as described under Procedure B. The catalyst and alcohol were removed and the residue of XIII condensed with 3.2 g. (0.02 mole) of alloxan monohydrate by the procedure of Lambooy.[19] One gram (16%) of IV was obtained, m.p. 214–216° dec.

Anal. Calcd. for $C_{18}H_{23}N_5O_4$: C, 57.9; H, 6.2; N, 18.8; Found: C, 57.6; H, 6.3; N, 18.6. ϵ_{max}[20] (λ) 265 (30,500), 370 (10,500), 455 (12,200); ϵ_{min} (λ) 240 (11,300), 300 (1,100), 400- (7,800). $R_f = 0.32$.

Procedure B is a modification of the procedure of Adams, *et al.*[2b] Five grams (0.017 mole) of XI in 150 ml. of absolute ethanol was reduced over platinum oxide at 3.5 kg./cm.^{2} for 17 hr. The catalyst was filtered and dry hydrogen chloride gas passed into the filtrate for a few min. The solution was concentrated to an oil and 50 ml. of absolute ethanol was added to dissolve the residue. To this solution of XIII hydrochloride was added a hot solution of 4 g. (0.02 mole) of alloxan monohydrate in 100 ml. of absolute ethanol. The resulting dark solution was heated for 10 min. on the steam bath and kept at room temperature overnight. The crude flavin hydrochloride was filtered and recrystallized from 775 ml. of 60% ethanol. After cooling, the product was collected and recrystallized from 150 ml. of 50% ethanol to give 3 g. (43%) of IV hydrochloride, m.p. 264–265° dec.

Anal. Calcd. for $C_{18}H_{24}ClN_5O_4$: Cl, 8.6. Found: Cl, 8.7.

7,8-Dichloro-10-{2-[bis(2-hydroxyethyl)amino]ethyl}isoalloxazine Hydrochloride (V).—Five grams (0.015 mole) of XII in 150 ml. of absolute ethanol was reduced over platinum oxide at 4.2 kg./cm.^{2} for 11 hr. The catalyst was filtered and dry hydrogen chloride gas passed into the filtrate. The alcohol was distilled and 50 ml. of absolute ethanol added to dissolve the residue. A hot solution of 4 g. (0.02 mole) of alloxan monohydrate in 150 ml. of absolute ethanol was added and the solution heated for 10 min. on the steam bath and let stand for 2 days in the dark at room temperature. The crude product was filtered and recrystallized from a mixture of 155 ml. of water, 10 ml. of concd. hydrochloric acid and 175 ml. of ethanol. The product was filtered and recrystallized from a mixture of 20 ml. of water, 5 ml. of concd. hydrochloric acid and 25 ml. of ethanol to give 0.87 g., m.p. 255–256° dec. A second crop of 0.23 g. was isolated from the filtrate, m.p. 253–255° dec.; total yield of V hydrochloride, 1.1 g. (13%). An analytical sample was obtained from 50% ethanol, m.p. 259–260° dec.

Anal. Calcd. for $C_{16}H_{18}Cl_3N_5O_3$: C, 42.6; H, 4.0; Cl, 23.6; N, 15.5. Found: C, 42.3; H, 4.0; Cl, 23.6; N, 15.4. ϵ_{max} (λ) 225 (32,400), 270 (34,000), 350 (8,500), 440 (12,000); ϵ_{min} (λ) 240 (22,400), 300 (2,000), 380 (4,700). $R_f = 0.44$.

7,8-Dimethyl-10-{2-[bis(2-chloroethyl)amino]ethyl}isoalloxazine Hydrochloride (VI).—The free base of IV hydrochloride was prepared by the neutralization of 3.33 g. (0.008 mole) of the hydrochloride with *N* sodium hydroxide to give 3.02 g. of IV, m.p. 210–212° dec. IV and 200 ml. of thionyl chloride were kept at room temperature for 12 hr. and refluxed for 2 hr. The thionyl chloride was distilled and 100 ml. of absolute ethanol added to the residue and distilled. The crystalline residue was transferred to a filter with the aid of 75 ml. of absolute ethanol. There was obtained 3.4 g. (91%) of VI hydrochloride, m.p. 252–253° dec. A 1.1 g. sample was recrystallized for analysis from a mixture of 20 ml. of concd. hydrochloric acid, 40 ml. of water and 80 ml. of ethanol. The product, m.p. 252–253°, was filtered and dried at 3 mm./100°/5 hr., m.p. 250–252° dec.

Anal. Calcd. for $C_{18}H_{22}Cl_3N_5O_2$: C, 48.4; H, 5.0; Cl, 23.8; N, 15.7. Found: C, 48.2; H, 5.1; Cl, 23.2, 23.4; N, 15.0, 15.1, 15.6.

7,8-Dichloro-10-{2-[bis(2-chloroethyl)amino]ethyl}isoalloxazine Hydrochloride (VII).—The free base of V hydrochloride was prepared by the neutralization of 1.23 g. (0.0027 mole) with *N* sodium hydroxide to give 1.1 g. of V, m.p. 202–204° dec. It was suspended in 200 ml. of thionyl chloride, let stand at room temperature for 15 hr. and then refluxed for 3 hr. After removal of the thionyl chloride, 100 ml. of absolute ethanol was distilled from the residue and the crystalline product was transferred to a filter with the aid of 60 ml. of absolute ethanol. The yield of VII hydrochloride was 1.16 g. (87%), m.p. 229–230° dec. A 0.54 g. sample was recrystallized from a mixture of 30 ml. of concd. hydrochloric acid and 45 ml. of ethanol. The product, m.p. 234–235° dec., was dried for analysis at room temperature and 3 mm. for 12 hr., m.p. 234–235° dec. Heating the sample during drying caused loss of hydrogen chloride.

Anal. Calcd. for $C_{16}H_{16}Cl_5N_5O_2$: C, 39.4; H, 3.3; Cl, 36.3; N, 14.3. Found: C, 39.3; H, 3.2; Cl, 36.6; N, 14.1.

(18) R. Kuhn, F. Weygand, and E. Möller, *Ber.*, **76**, 1044 (1943).
(19) J. P. Lambooy, *J. Am. Chem. Soc.*, **72**, 5225 (1950).
(20) ϵ Has units of l./mole-cm.

Vitamin B_6 Analogs. 1. 5-Hydroxy-6-methyl-4-trifluoromethyl-3-pyridinemethanol Hydrochloride[1]

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A total synthesis of 5-hydroxy-6-methyl-4-trifluoromethyl-3-pyridinemethanol hydrochloride is described. The condensation of trifluoroacetylacetone with 2-cyanoacetamide leads to 2-hydroxy-6-methyl-4-trifluoromethylnicotinonitrile. This pyridine is then converted to the final product by a five-step reaction sequence.

Although Stoerk[2] observed in 1947 that 4-desoxypyridoxine administered to hybrid mice maintained on a vitamin B_6-deficient diet inhibited the growth of lymphosarcoma 6C3H-ED, relatively little work has been done on the syntheses of other analogs of the B_6 vitamins for screening against animal neoplasms. Other workers have investigated the effect of 4-desoxypyridoxine and acid hydrazides, separately and in combination, on the growth of other rodent tumors.[3] In all cases the treatments were significantly more effective on a B_6-deficient diet.

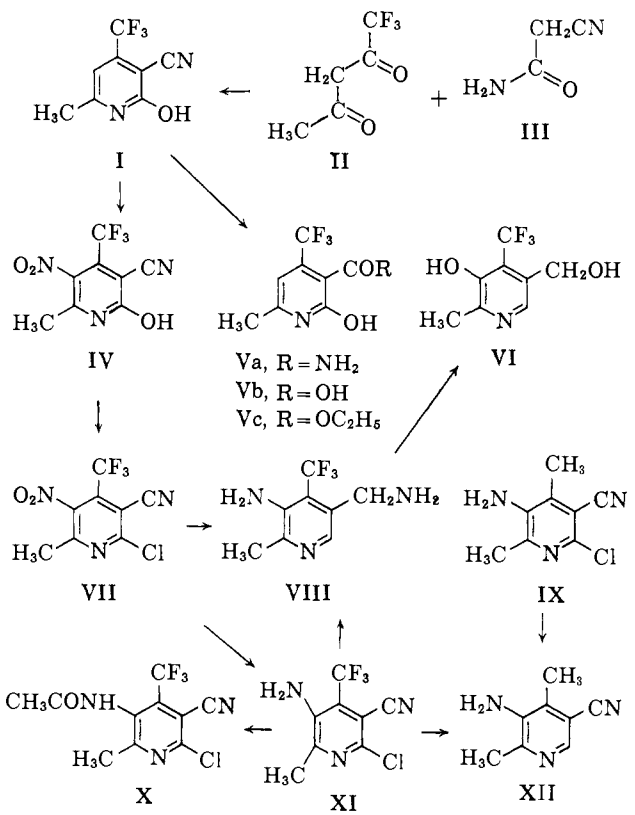
(1) This work was supported by funds from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740.

(2) H. C. Stoerk, *J. Biol. Chem.*, **171**, 437 (1947).

(3) D. M. Shapiro and A. Gellhow, *Cancer Research*, **11**, 35 (1951); R. R. Cerecedo, M. E. Lombardo, D. V. M. Reddy, and J. J. Travers, *Proc. Soc. Exper. Biol. Med.*, **80**, 648 (1952); B. L. Freedlander, F. A. French, and A. Furst, *ibid.*, **86**, 788 (1954); J. B. Loefer, *Cancer Research*, **11**, 481 (1950); and R. W. Brockman, J. R. Thomson, F. M. Schabel, Jr., and H. E. Skipper, *ibid.*, **16**, 788 (1956).

As a part of our program to develop more effective antineoplastic agents, we have undertaken the syntheses of a number of analogs of the B_6 vitamins with the objective in mind of obtaining a compound with significant antitumor activity on a complete diet.

One of our first candidates for synthesis and evaluation was "trifluoro-4-desoxypyridoxine" [5-hydroxy-6-methyl-4-(trifluoromethyl)-3-pyridinemethanol (VI)]. Since the atomic radius of fluorine is only slightly larger than that of hydrogen, this compound should fulfill the structural requirements of an antagonist of the B_6 vitamins,[4] but, because of the powerful inductive effect of the trifluoromethyl group, should possess chemical properties quite different from those of B_6 vitamins. For example, one would expect the 5-hydroxyl group of VI to be more acidic than the corresponding hydroxyl of the B_6 vitamins and the ring nitrogen of VI to be less basic than that of the B_6 vitamins.[5] Since it is not possible to predict the effect of such differences on the biologic activity of VI, synthesis of this compound for biological evaluation seemed worthwhile. The syntheses of a number of compounds closely related to



pyridoxine have been reported in the literature. None of these substances, however, contain trifluoromethyl substituents. The condensations of 2-cyanoacetamide with acetylacetone, with 1-ethoxyacetylacetone, and with ethyl acetopyruvate lead, respectively, to 4,6-dimethyl-2-hydroxynicotinonitrile,[8] 4-ethoxymethyl-2-hydroxy-6-methylnicotinonitrile,[9] and 4-carbethoxy-2-hydroxy-6-methylnicotinonitrile.[10] Therefore, we believed that the condensation of 1,1,1-trifluoroacetylacetone (II) with 2-cyanoacetamide (III) would lead to 2-hydroxy-6-methyl-4-(trifluoromethyl)-nicotinonitrile (I). A single product was isolated when the condensation was effected in ethanol using the general procedure described by Verrill and Schneider.[11] While no formal proof of the position of the trifluoromethyl group on the pyridine ring was attempted (it could occupy either the 2- or the 4-position), early work of Bardhan,[12] and later that of Wenner and Plati,[13] indicates that the trifluoromethyl group is in the 4-position.

Acid hydrolysis of the nitrile gave the corresponding amide (Va), while basic hydrolysis and acidification gave the free acid (Vb). The latter was converted to the ethyl ester (Vc) in excellent yields by a modified Fischer esterification. Nitration of the 2-hydroxy-6-methyl-4-(trifluoromethyl)nicotinonitrile (I) with fuming nitric acid in sulfuric acid solution gave 2-hydroxy-6-methyl-5-nitro-4-(trifluoromethyl)nicotinonitrile (IV). Treatment of this nitro pyridine (IV) with phosphorus pentachloride in phosphorus oxychloride gave high yields of 2-chloro-6-methyl-5-nitro-4-(trifluoromethyl)nicotinonitrile (VII). This compound (VII) when treated with hydrogen in the presence of palladium-on-carbon catalyst could be converted directly to 3-amino-5-aminomethyl-2-methyl-4-(trifluoromethyl)pyridine (VIII) trihydrochloride. When, however, reduction of the nitro group, removal of the halogen, and reduction of the cyano group were all effected in one step, the yield was poor. If the nitro group of VII was first reduced with stannous chloride and hydrochloric acid[14] to give 5-amino-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile (XI) and XI in turn reduced catalytically to the diamine VIII, the over-all yield was much better. The intermediate XI was characterized as its acetyl derivative (X). Because the free base XI precipitated from an acid medium, a potentiometric titration of XI in aqueous solution was carried out. No break in the titration curve was observed confirming the weakly basic nature of the 5-amino group of XI, apparently resulting from the combined inductive effects of the trifluoromethyl group and the nitrile group.

An attempt to remove the 2-chloro group from compound XI chemically gave an unexpected and interesting result. In some related work which will be reported in a subsequent paper we had been able to convert 5-amino-2-chloro-4,6-dimethylnicotinonitrile (IX) to 5-amino-4,6-dimethylnicotinonitrile (XII) by prolonged boiling of IX with an aqueous suspension of zinc dust. When this procedure was carried out on the corresponding 4-trifluoromethyl compound (XI) the chlorine was readily removed, but at the same time all three fluorine atoms of the 4-trifluoromethyl group were also replaced by hydrogen; the fluorine-free compound (XII) was obtained in essentially quantitative

(4) E. E. Snell, *Vitamins and Hormones*, **16**, 77 (1958).

(5) Giner-Sorolla and Bendich found that the introduction of the 6-trifluoromethyl group into uracil caused a 6,000-fold increase in the acid strength of uracil.[6] The introduction of a single fluorine atom into the 2- or 3-position of pyridine brings about a similar decrease in basic strength of the ring nitrogen.[7]

(6) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 5744 (1958).

(7) H. C. Brown and D. H. McDaniel, *ibid.*, **77**, 3752 (1955).

(8) J. Moir, *J. Chem. Soc.*, **81**, 105 (1902).

(9) S. A. Harris, E. T. Stiller, and K. Folkers, *J. Am. Chem. Soc.*, **61**, 1242 (1939).

(10) A. Itiba and S. Emoto, *Sci. Papers Inst. Phys. Chem. Research* (Tokyo) **38**, 347 (1941) [*C. A.*, **35**, 6960 (1941)].

(11) K. J. Verrill and A. M. Schneider, British Patent 686,012 (1953).

(12) J. C. Bardhan, *J. Chem. Soc.*, 2223 (1929).

(13) W. Wenner and J. T. Plati, *J. Org. Chem.*, **11**, 751 (1946).

(14) L. A. Perez-Medina, R. P. Mariella, and S. M. McElvain, *J. Am. Chem. Soc.*, **69**, 2574 (1947).

TABLE I

Compound	Dosage (mg./kg./day)	Tumor	Mortality	Animal wt. change T/C (g.)	Av. tumor wt. T/C (mg.)	Av. tumor wt. % of Control
I	500	S180	1/6	−0.5/+1.7	652/857	76
	400	Ca755	1/10	+1.7/+2.1	1291/1120	>100
IV	500	S180	6/6			
	100	S180	0/6	+1.6/+2.1	1673/1915	87
	90	Ca755	0/10	+1.0/+1.3	980/1204	81
VI	500	S180	6/6			
	100	S180	1/6	+0.3/+0.5	1245/1147	>100
	80	Ca755	10/10			
	40	Ca755	1/10	+1.2/+3.2	984/1709	57
VII	62	S180	6/6			
	15	S180	0/6	−1.6/+0.2	1136/1704	70
XI	500	S180	1/6	−0.2/+1.7	490/857	57
	400	Ca755	10/10			

yield. This degree of lability of the fluorine atoms in a trifluoromethyl group is quite unusual and unexpected.[15]

5-Amino-2-chloro-6-methyl-4-(trifluoromethyl)-nicotinonitrile (XI), when treated with three equivalents of hydrogen at atmospheric pressure in the presence of 30% palladium-on-carbon catalyst, gave essentially quantitative yields of 3-amino-5-aminomethyl-2-methyl-4-(trifluoromethyl)pyridine (VIII) isolated as the dihydrochloride. Attempts to effect this same reduction with lithium aluminum hydride were not successful. No identifiable product was isolated; partial reduction of the pyridine ring appeared to take place. This result is in agreement with those obtained by Bohlmann,[17] who found that many pyridines substituted in the 3-and/or 5-position by electron withdrawing groups are subject to ring reduction by lithium aluminum hydride.

Reaction of 3-amino-5-aminomethyl-2-methyl-4-(trifluoromethyl)-pyridine (VIII) dihydrochloride or trihydrochloride with nitrous acid gave moderate yields of the desired pyridoxine analog, 5-hydroxy-6-methyl-4-(trifluoromethyl)-3-pyridinemethanol (VI) hydrochloride.

Screening Results.—"Trifluoro-4-desoxypyridoxine" (VI) and most of the synthetic intermediates leading to it were screened against Sarcoma 180 and Adenocarcinoma 755.[18] The results given in Table I show that none of these compounds has significant activity against either one of these tumors.

These compounds were also evaluated for anti-B_6 activity in *Saccharomyces carlsbergensis* by a procedure already described.[19] Compounds I, IV, and VI exhibited no inhibition at a concentration of 3 μg./ml. and hence, by comparison with 4-deoxypyridoxine, may be considered inactive as B_6 antagonists. Compound XI inhibited growth to the same extent in concentrations ranging from 0.1 μg./ml. to 3.0 μg./ml. indicating that its inhibitory action is not solely (if at all) a result of B_6 antagonism. Expressed in terms of anti-B_6 activity, this compound has one-third the activity of 4-deoxypyridoxine on a weight basis at a concentration of 0.1 μg./ml.

Experimental

All melting points were determined in an open capillary and are corrected.

2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinonitrile (Ia).—2-Cyanoacetamide (47 g., 0.56 mole) was dissolved in boiling 95% ethanol (350 ml), 1,1,1-trifluoroacetylacetone[20] (77 g., 0.50 mole) added, and the system gently refluxed with stirring while diethylamine (18.3 g., 0.25 mole) was added dropwise over a 30 min. period. Refluxing and stirring were continued for an additional hr. before the system was allowed to stand overnight at 0°. The yellow crystals that had formed were collected on a filter and dried at 80°; yield 81 g. (80%); m.p. 236.5–238.5°. Recrystallization from ethanol did not raise the melting point.

Anal. Calcd. for $C_8H_5F_3N_2O$: C, 47.76; H, 2.51; N, 13.93. Found: C, 47.76; H, 2.73; N, 13.89.

2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinamide (Va).—2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinonitrile (4.66 g.) was dissolved in a mixture of sulfuric acid (25 g., sp. gr. 1.84) and fuming sulfuric acid (23 g., 20% SO_3). The solution was allowed to stand at 80° for 24 hr. before being poured into an ice-water slush (300 ml.). The white, crystalline precipitate was collected on a filter and dried before being recrystallized from ethanol; yield 3.67 g. (72%); m.p. 341.5–343.5° dec.

Anal. Calcd. for $C_8H_7F_3N_2O_2$: C, 43.63; H, 3.21; F, 25.90; N, 12.72. Found: C, 43.88; H, 3.30; F, 25.9; N, 12.71.

2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinic Acid (Vb).—2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinonitrile (10 g.) was added to a 25% solution (110 ml.) of potassium hydroxide in water, and the system was refluxed gently for 18 hr. before being poured into an ice-water slush (300 ml.) containing concd. hydrochloric acid (60 ml.). The white, crystalline precipitate was collected on a filter and dried before being recrystallized from 150 ml. of 50% acetic acid; yield 7.5 g. (69%); m.p. 250–251°.

Anal. Calcd. for $C_8H_6F_3NO_3$: C, 43.46; H, 2.74; N, 6.34. Found: C, 43.67; H, 2.97; N, 6.38.

Ethyl 2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinate (Vc). A mixture of 2-hydroxy-6-methyl-4-(trifluoromethyl)nicotinic acid (44.6 g., 0.202 mole), absolute ethanol (55.8 g., 1.21 moles), sodium chloride (23.6 g., 0.404 mole), benzene (250 ml.), and concd. sulfuric acid (41.7 g., 0.404 mole) was refluxed vigorously until no more water collected in the attached water trap (60 hr.). After cooling, the contents of the flask were made basic with concd. ammonium hydroxide and extracted with an equal volume of chloroform. The organic layer was separated and the aqueous layer extracted a second time with chloroform (250 ml.). The original organic layer and chloroform extract were combined, washed with water, and dried over Drierite. After removal of the desiccant by filtration the solvent was evaporated *in vacuo* to give a white, crystalline residue which was recrystallized from water (1500 ml.); yield 34.5 g. (69%); m.p. 142–144°.

Anal. Calcd. for $C_{10}H_{10}F_3NO_3$: C, 48.19; H, 4.05; N, 5.62. Found: C, 48.24; H, 4.12; N, 5.58.

(15) Another interesting exception to the normal stability of the trifluoroethyl group has been noted recently. 5-Trifluoromethyluracil is hydrolyzed readily in base to the corresponding carboxylic acid.[16]

(16) C. Heidelberger, D. Parsons, and D. C. Remy, *J. Am. Chem. Soc.*, **84**, 3597 (1962).

(17) F. Bohlmann and M. Bohlmann, *Chem. Ber.*, **86**, 1419 (1953).

(18) *Cancer Chemotherapy Reports*, **1**, 42 (1959).

(19) E. E. Snell and J. C. Rabinowitz, *Anal. Chem.*, **19**, 277 (1947).

(20) Columbia Organic Chemicals Co., Inc., Columbia, South Carolina.

2-Hydroxy-6-methyl-5-nitro-4-(trifluoromethyl)nicotinonitrile (IV).—2-Hydroxy-6-methyl-4-(trifluoromethyl)nicotinonitrile (50 g.) was dissolved in concd. sulfuric acid (140 ml.). To this solution, cooled to 0°, was added with thorough mixing a solution (also at 0°) of fuming nitric acid (120 ml., *d* 1.5) in concd. sulfuric acid (250 ml.). The mixture was stirred constantly while the temperature rose spontaneously to 45°. When the temperature had fallen to 35° the mixture was cooled to 15° and poured with vigorous stirring into crushed ice (2000 ml.). The fine, yellow precipitate was collected on a filter and washed with water before being dried at 80°. The crude product was dissolved in boiling ethanol (300 ml.) and treated with hot water to the point of first permanent cloudiness. After standing overnight at 0° the yellow crystals were collected and dried; yield 44 g. (72%); m.p. 202–204° dec.

Anal. Calcd. for $C_8H_4F_3N_3O_3$: C, 39.88; H, 1.63; N, 17.00. Found: C, 39.49; H, 1.74; N, 17.00.

2-Chloro-6-methyl-5-nitro-4-(trifluoromethyl)nicotinonitrile (VII).—2-Hydroxy-6-methyl-5-nitro-4-(trifluoromethyl)-nicotinonitrile (75 g., 0.30 mole), phosphorus pentachloride (94 g., 0.45 mole), and phosphorus oxychloride (250 ml.) were mixed and heated at gentle reflux for 18 hr. At the end of this time essentially all of the phosphorus oxychloride was removed by vacuum distillation. The residue was chilled and treated with an ice-cold mixture of ethanol (100 ml.) and water (400 ml.). After the reaction had subsided the mixture was heated for 2 min. on a steam bath before being cooled in ice. The precipitate was collected on a filter, washed thoroughly with water and dried on the filter before being recrystallized from aqueous ethanol; yield after recrystallization 66 g. (81%); m.p. 96–97°.

Anal. Calcd. for $C_8H_3ClF_3N_3O_2$: C, 36.18; H, 1.14; N, 15.82. Found: C, 36.19; H, 1.21; N, 15.63.

5-Amino-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile (XI).—2-Chloro-6-methyl-5-nitro-4-(trifluoromethyl)nicotinonitrile (35 g.) was slurried with diethyl ether (100 ml.) contained in a 2 l. flask. A clear solution of stannous chloride (90 g.) in concd. hydrochloric acid (180 ml.) was added to the stirred slurry. Immediate exothermic reaction drove off most of the ether, and stirring was continued until the reaction temperature had fallen to 30°. Ice water (350 ml.) was added, and the system was allowed to stand at 0° overnight before the crude product was collected on a filter and washed with two 100-ml. portions of 5% hydrochloric acid followed by 200 ml. of water. The crude product was dried at 80° and recrystallized twice from aqueous ethanol; yield 25 g. (78%); m.p. 134–136°.

Anal. Calcd. for $C_8H_5ClF_3N_3$: C, 40.78; H, 2.14; N, 17.84. Found: C, 40,84; H, 2.26; N, 17.58.

5-Acetamido-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile (X).—This derivative was prepared from 5-amino-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile (2 g.) *via* a standard procedure[21]; yield 2.15 g. (91%); m.p. 193.5–195.5°.

Anal. Calcd. for $C_{10}H_7ClF_3N_3O$: C, 43.26; H, 2.55; N, 15.14. Found: C, 43.17; H, 2.75; N, 15.27.

Reduction of 5-Amino-2-chloro-6-methyl-4-(trifluoromethyl)-nicotinonitrile: 5-Amino-4,6-dimethylnicotinonitrile (XII).—(a) 5-Amino-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile (2.0 g.), water (200 ml.), and zinc dust (20 g.) were heated together at gentle reflux for 1 hr. More zinc dust (5 g.) was added, and refluxing was continued for 71 hr. The hot solution was filtered quickly and the filtrate reheated to boiling to give a clear water-white solution which was allowed to stand overnight at 0°. The white silky needles were collected on a filter, washed with water, and dried at 80°; yield of fluorine-free compound 1.1 g. (88%); m.p. 193.5–195.5°.[22]

Anal. Calcd. for $C_8H_9N_3$: C, 65.25; H, 6.16; N, 28.55. Found: C, 65.58; H, 6.27; N, 28.38.

(b) When an attempt was made to reduce 5-amino-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile with lithium aluminum hydride in diethyl ether solution, work-up gave an oil possessing a strong ammoniacal odor. This oil could not be induced to crystallize nor could an isolable hydrochloride salt be obtained from it.

(c) **Catalytic Reduction of XI: 3-Amino-5-aminomethyl-6-methyl-4-(trifluoromethyl)pyridine (VIII) Dihydrochloride.**—5-Amino-2-chloro-6-methyl-4-(trifluoromethyl)nicotinonitrile (10 g.) was suspended in water (200 ml.) containing concd. hydrochloric acid (51 ml.) and 30% palladium-on-carbon catalyst (10 g.). The system was stirred and treated with hydrogen at essentially atmospheric pressure until 3 equiv. of the gas had been absorbed (actual uptake was 3150 ml.; theoretical uptake at 750 mm. and 25°, 3140 ml.). The system was heated to boiling, filtered hot, and the filter cake washed with hot 5% hydrochloric acid (100 ml). The combined filtrates were evaporated to dryness on a steam bath, leaving a yellow, crystalline residue that was slurried with boiling ethanol (200 ml.) in which it was not completely soluble. After cooling, the suspension was treated with an equal volume of diethyl ether and chilled to 0° before the precipitate was collected on a filter; yield 10.8 g. (92%); m.p. 217–218° dec. The apparent pK_a values of this compound, determined potentiometrically, are 3.35 and 8.80; neutral equivalent: calcd., 138.6; found, 138.1.

Anal. Calcd. for $C_8H_{10}F_3N_3 \cdot 2HCl$: C, 34.55; H, 4.35; Cl, 25.50. Found; C, 34.28; H, 4.56; Cl, 25.80.

3-Amino-5-aminomethyl-6-methyl-4-(trifluoromethyl)pyridine (VIII) Trihydrochloride.—2-Chloro-6-methyl-5-nitro-4-(trifluoromethyl)nicotinonitrile (10 g.), water (200 ml.), concd. hydrochloric acid (15 ml.), and 30% palladium-on-carbon catalyst (10 g.) were treated with hydrogen at 3.5 kg./cm.^{2} When pressure drop indicated that 6 equiv. of hydrogen had been absorbed (28 hr.), the system was opened, the reaction mixture heated to boiling and filtered hot. The clear yellow filtrate was evaporated to dryness, and the residue was recrystallized from ethanol; yield 5.5 g. (46%); m.p. 276–278° dec.

Anal. Calcd. for $C_8H_{10}F_3N_3 \cdot 3HCl$: C, 30.54; H, 4.16; N, 13.36. Found: C, 30.92; H, 4.24; N, 13.54.

5-Hydroxy-6-methyl-4-(trifluoromethyl)-3-pyridinemethanol (VI) Hydrochloride.—3-Amino-5-aminomethyl-2-methyl-4-(trifluoromethyl)-pyridine dihydrochloride (8 g.) was dissolved in water (200 ml.) containing concd. hydrochloric acid (11 ml.). This solution was maintained at 80 ± 3° and stirred while a solution of sodium nitrite (4.4 g.) in water (20 ml.) was added dropwise over a 1.5-hr. period. Temperature and stirring then were maintained for an addition 0.5 hr. The yellow solution was evaporated to dryness on a steam bath; the residue was taken in boiling ethanol (100 ml.), treated with decolorizing carbon and filtered hot. The filtrate was treated with acetone (100 ml.) and allowed to stand at 0° for 3 hr. before the small amount of precipitate was removed by filtration. The filtrate was evaporated to dryness *in vacuo*, and the residue dissolved in absolute ethanol (60 ml.). The clear, pale yellow solution was treated with anhydrous ether (150 ml.) and allowed to stand overnight at 0° before the white crystalline precipitate was collected on a filter, washed with ether, and dried at 78° *in vacuo;* yield 5.1 g. (73%); m.p. 192.5–194.5° dec.

Anal. Calcd. for $C_8H_8F_3NO_2 \cdot HCl$: C, 39.44; H, 3.72; N, 5.75. Found: C, 39.28; H, 3.82; N, 5.96.

The same reaction was carried out on 3-amino-5-aminomethyl-2-methyl-4-trifluoropyridine trihydrochloride (see above) and gave material identical with that described above; m.p. 192–193°, mixture melting point with the material described above, 192–193°. Both samples of VI gave a positive test for ionic chloride.

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(21) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," J. Wiley and Sons, Inc., New York, N. Y., 3rd ed. 1948, p. 177.

(22) This melting point is identical with and does not depress that of an authentic sample of 5-amino-4,6-dimethylnicotinonitrile prepared in an unambiguous manner which will be described in a subsequent paper of this series.