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SYNTHETIC TUBERCULOSTATS. I. PYRIDINE CARBOXYLIC ACID DERIVATIVES

H. HERBERT FOX

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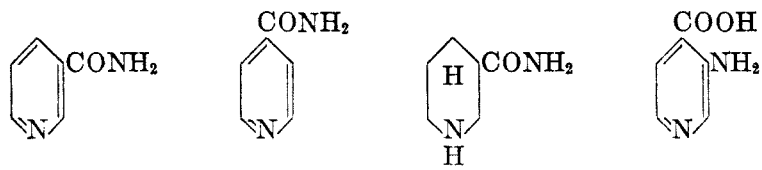
The tuberculostatic activity of nicotinamide and some nicotinamide derivatives in mice (1, 2, 3) suggested the desirability of investigating other closely related compounds in the hope that some pattern of structure-activity relationship might be uncovered which would lead to compounds of much greater activity. As the work progressed, it became apparent that the desired structure-activity pattern would not emerge. In fact, none of the compounds synthesized in the early part of this study showed any activity at all. The scope of the work was therefore enlarged to include the pyridine analog of *p*-aminosalicylic acid and some of its derivatives.

The anti-tubercular activity of the vitamin, nicotinamide, was first discovered in France in 1945 by Chorine (1). He also showed that nicotinic acid, despite its vitamin activity, was not tuberculostatic and therefore justly contended that there was no relationship between the two activities. Unfortunately, this very significant discovery appeared to attract no attention, since it was rediscovered in this country in 1948 by McKenzie, *et al.* (3) who postulated that the tuberculostatic effect of nicotinamide and some of its derivatives was a function of their vitamin activity. For example, they found that, when one of the hydrogens of the amide grouping in nicotinamide was replaced by an isopropyl, pyridyl, or thiazolyl group, the tuberculostatic activity, though diminished, was still retained. On the other hand, placement of another substituent, such as Cl, NH_2, or C_4H_9O in the 6-position on the ring completely abolished the activity of nicotinamide. It appeared, therefore, that the role of nicotinamide in the treatment of mouse tuberculosis was that of a vitamin. This concept was borne out by the initial results of this study when, for example, compounds as closely related structurally to nicotinamide (I) as isonicotinamide (II) and nipecotamide (III) proved to be totally inactive.



I II III IV

Subsequently, however, the postulate was negated by the marked activity demonstrated by 3-aminoisonicotinic acid (IV). This latter compound, which had no vitamin activity, not only had a carboxyl group in a different position but also had another substituent on the ring. Despite these two pronounced deviations from those structures which were associated with activity, 3-amino-

isonicotinic acid and its methyl ester proved to be about one-half as active as nicotinamide itself. This suggested the possibility that tuberculostatic activity might exist in a wide variety of pyridine compounds.

The compounds prepared in this study are listed in Table I.

TABLE I

PYRIDINE CARBOXYLIC ACID DERIVATIVES

Nucleus	SUBSTITUENT 2	3	4	5	6	SALT	M.P., °C. CORR.	REF.	ACTIVITY
R	CH_3	$CONH_2$	C_6H_5		CH_3	—	200	(4)	0
R	CH_3	$CONH_2$		$CONH_2$	CH_3	—	300	E	
R	CH_3	$CONH_2$		$CONH_2$	CH_3	HCl	300	E	0
R	CH_3	$CONH_2$		$COOC_2H_5$	CH_3	—	159–160.5	E	0
R	$CONH_2$					—	108–109	(5)	0
R	$CONH_2$					HCl	212	(6)	
R			$CONH_2$			HCl	262–265		0
R			$CONH_2$			—	155–156	(7)	
R		OH	COOH			—	312	(8)	0
R		NH_2	COOH			—	300	(9)	+
R		CH_3CONH	COOH			—	266–267	E	0
R		NH_2CONH	COOH			—	300	E	0
R		NH_2	$COOCH_3$			—	86–88	(9)	+
R		NH_2	$CONH_2$			—	151–152	E	0
R′		$CONH_2$				$H_2C_2O_4$	203–205	E	0
R′		$CONH_2$				HCl	244–246	E	
R′		$CONH_2$				—	111–112	E	
R′			$CONH_2$			HCl	215–217	E	0
R′			$CONH_2$			—	152–153	E	
R′	$CONH_2$					—	147	E	0
R′	$CONH_2$					HCl	254	E	

E = Experimental

The experimental details are given only for those compounds which have not appeared in the literature.

Under the column headed "Activity" only the presence or absence of activity is noted and no attempt is made to indicate the degree of activity. Actually though, as was indicated before, the activities of 3-aminoisonicotinic acid and its methyl ester in mouse infections are approximately half that of nicotinamide itself.

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operation in evaluating the compounds prepared in this study. The author also wishes to acknowledge his indebtedness to Dr. A. Steyermark and his staff for the microanalyses, and to Mr. J. I. Lewis for his technical assistance in preparing some of the compounds.

EXPERIMENTAL

All melting points are corrected.

2,6-Dimethyl-3,5-pyridinedicarboxamide and 2,6-dimethyl-5-carbethoxynicotinamide. A mixture of 10 g. of 2,6-dimethyl-3,5-dicarbethoxypyridine and 70 cc. of liquid ammonia in ethanol was heated at 130° under 500 lbs. nitrogen pressure for about 20 hours. The reaction mixture was filtered to remove the fine powdery precipitate of 2,6-dimethyl-3,5-pyridinedicarboxamide which was purified by solution in hot, dilute acetic acid and reprecipitation with dilute ammonium hydroxide. Small, colorless needles. Dec. >300°. Insoluble in organic solvents; slightly soluble in hot water.

Anal. Calc'd for $C_9H_{11}N_3O_2$: C, 56.0; H, 5.7.

Found: C, 55.6; H, 5.8.

The filtrate remaining after removal of the diamide was diluted with water to give long, fine, white needles of 2,6-dimethyl-5-carbethoxynicotinamide; m.p. 159–160.5° on recrystallization from water.

Anal. Calc'd for $C_{11}H_{14}N_2O_3$: C, 59.5; H, 6.3.

Found: C, 59.5; H, 6.5.

2,6-Dimethyl-3,5-pyridinedicarboxamide hydrochloride. A portion of the free base was suspended in water, and 2 *N* sulfuric acid was added to dissolution of the base. On treatment of the solution with an excess of dilute hydrochloric acid, the diamide hydrochloride precipitated. The hydrochloride was purified by solution in hot water and reprecipitation with a little dilute hydrochloric acid. Small crystal clusters. Dec. > 300°.

Anal. Calc'd for $C_9H_{11}N_3O_2 \cdot HCl$: N, 18.3. Found: N, 18.8.

3-Acetamidoisonicotinic acid. A mixture of 4 g. of 3-aminoisonicotinic acid, 2 g. of anhydrous sodium acetate, and 20 cc. of acetic anhydride was refluxed for about 1 hour. The acetic acid and acetic anhydride were removed under a vacuum, and the residue was treated with water. On cooling and standing, 4.2 g. of the acetyl compound was obtained. Pale yellow plates from water; m.p. 266–267°.

Anal. Calc'd for $C_8H_8N_2O_3$: C, 53.3; H, 4.4.

Found: C, 53.3; H, 4.4.

3-Ureidoisonicotinic acid. A mixture of 13.8 g. (0.1 mole) of 3-aminoisonicotinic acid and 16.2 g. (0.2 mole) of potassium cyanate in 200 cc. of water was kept at 45° until solution was complete. The mixture was cooled, and a slight excess of glacial acetic acid was added, upon which 18 g. of the ureido compound precipitated. It formed small, white crystals by reprecipitation from hot, dilute ammonium hydroxide with glacial acetic acid; m.p. >300° Soluble in alkalis and dilute hydrochloric acid; insoluble in water, dilute acetic acid and organic solvents.

Anal. Calc'd for $C_7H_7N_3O_3$: N, 23.2. Found: N, 23.8.

3-Aminoisonicotinamide. A mixture of 1 g. of methyl 3-aminoisonicotinate, 50 cc. of methanol, and 10 cc. of liquid ammonia was heated at 80–100° for about 4 hours in an autoclave under about 500 lbs. nitrogen pressure. The methanol was then removed under a vacuum to give an oil which solidified on cooling and scratching. Small, pale yellow crystals from benzene; m.p. 151–152°. Very soluble in acetone, water and alcohol; soluble in ethyl and butyl acetate; insoluble in cold benzene.

Anal. Calc'd for $C_6H_7N_3O$: C, 52.6; H, 5.1.

Found: C, 52.8; H, 5.2.

Nipecotamide oxalate. Nicotinamide (6 g.) in dry dioxane was hydrogenated at 150° and 200 lbs. pressure using Raney nickel as catalyst. The dioxane was removed under a vacuum, and the waxy residue was dissolved in methanol and treated with an excess of

oxalic acid in methanol to give a precipitate of nipecotamide oxalate. The product was purified by solution in a little water and reprecipitation with excess ethanol; colorless needles, dec. 203–205°.

Anal. Calc'd for $C_6H_{12}N_2O \cdot H_2C_2O_4$: C, 44.0; H, 6.4; N, 12.8.
Found: C, 44.3; H, 6.7; N, 12.7.

Nipecotamide hydrochloride was obtained by the reduction of nicotinamide hydrochloride with hydrogen using platinum as catalyst and glacial acetic acid as solvent at 50° and 50 lbs. pressure. On treating the reaction mixture with ether, the product precipitated out and was recrystallized from dilute alcohol. Colorless crystal clusters; m.p. 244–246°. Very soluble in water; insoluble in absolute alcohol and ether.

Anal. Calc'd for $C_6H_{12}N_2O \cdot HCl$: C, 43.8; H, 7.9.
Found: C, 44.0; H, 8.1.

The *free base* was obtained by treating an aqueous solution of the hydrochloride with freshly prepared silver oxide, filtering off the silver chloride and excess oxide, and evaporating the filtrate to dryness. The crude base was then recrystallized from a mixture of chloroform and Skellysolve "B" to give colorless needles and plates melting at 111–112°. The base is very soluble in water, alcohol, and chloroform; slightly soluble in hot benzene and ethyl acetate; insoluble in diethyl ether and petroleum ether.

Anal. Calc'd for $C_6H_{12}N_2O$: C, 56.2; H, 9.4.
Found: C, 56.2; H, 9.2.

Isonipecotamide hydrochloride. Isonicotinamide hydrochloride (2.5 g.) in methanol was quantitatively reduced to isonipecotamide hydrochloride with hydrogen and a platinum catalyst at about 50° and 50 lbs. pressure. The product was recrystallized from a mixture of methanol and 2-propanol. Colorless crystals; m.p. 215–217°.

Anal. Calc'd for $C_6H_{12}N_2O \cdot HCl$: C, 43.8; H, 7.9.
Found: C, 43.5; H, 8.0.

The *free base* was obtained from the hydrochloride with silver oxide. Fine, colorless needles from a chloroform-benzene mixture; m.p. 152–153°. Soluble in alcohol, water, and hot chloroform; slightly soluble in hot benzene and ethyl acetate; insoluble in diethyl ether.

Anal. Calc'd for $C_6H_{12}N_2O$: C, 56.2; H, 9.4.
Found: C, 56.0; H, 9.1.

Pipecolamide. Picolinamide hydrochloride (3.2 g.) in glacial acetic acid was quantitatively reduced with hydrogen and platinum catalyst at about 50° and 50 lbs. pressure to pipecolamide hydrochloride. The hydrochloride melted (with previous darkening) at 254° after recrystallization from a methanol-2-propanol mixture.

Anal. Calc'd for $C_6H_{12}N_2O \cdot HCl$: C, 43.8; H, 7.9.
Found: C, 44.0; H, 7.7.

A portion of the hydrochloride was converted to the free base with sodium hydroxide solution. On recrystallization from benzene, the *free base* was obtained in the form of small, white crystals melting at 147°.

Anal. Calc'd for $C_6H_{12}N_2O$: C, 56.3; H, 9.4.
Found: C, 56.9; H, 9.4.

CONCLUSION

The discovery of the marked tuberculostatic activity of 3-aminoisonicotinic acid demonstrated that the activity of nicotinamide and some of its derivatives was not necessarily related to their vitamin function. Moreover, it suggested the possibility that tuberculostatic activity might exist in a wide variety of pyridine compounds.

On the basis of the very limited data so far available, it would appear that any change made in the 3-position of 3-aminoisonicotinic acid, such as reduction, hydrolysis, or acylation completely supresses activity.

SUMMARY

A series of compounds was prepared and investigated for tuberculostatic activity. 3-Aminoisonicotinic acid and its methyl ester showed distinct activity. All the other compounds were inactive.

NUTLEY 10, N. J.

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