[CONTRIBUTION NO. 308 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE INC.]

SYNTHETIC TUBERCULOSTATS. IV. PYRIDINE CARBOXYLIC ACID HYDRAZIDES AND BENZOIC ACID HYDRAZIDES

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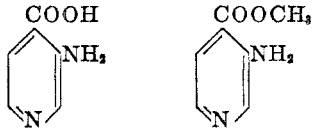
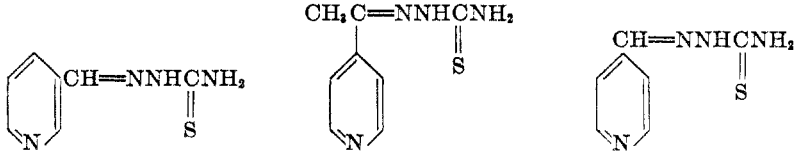
As the result of a systematic investigation in this laboratory into the anti-tubercular activity of pyridine compounds in general and pyridine carboxylic acid derivatives in particular, some interesting observations were made which paved the way to broader exploration in the field. Perhaps the most significant of these observations was the discovery of the tuberculostatic activity of 3-aminoisonicotinic acid (I) and its methyl ester (II) (1). These were the first



I II

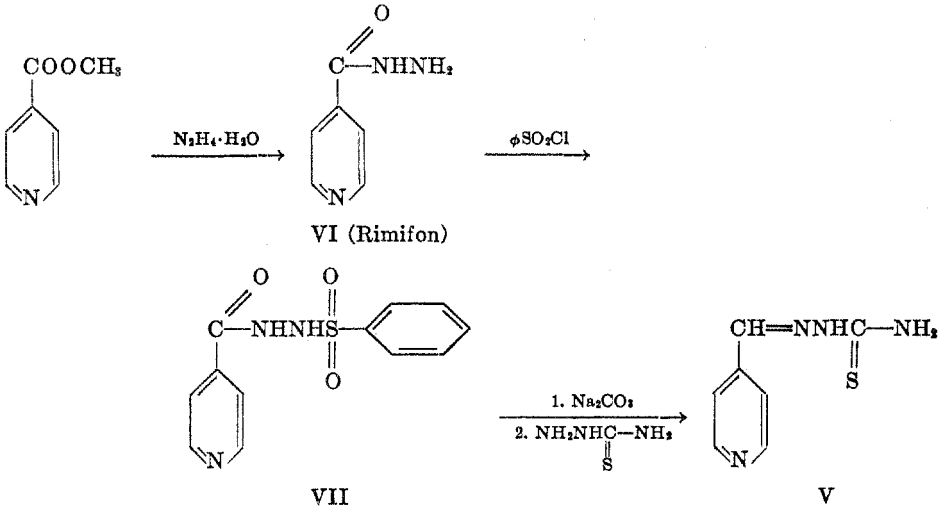
pyridine compounds, other than nicotinamide and its immediate derivatives, which, to our knowledge, showed any *in vivo* anti-tubercular activity. Though both of these compounds (I and II) were only about ½ as active as nicotinamide and were of no interest clinically, they did serve to show that anti-tubercular activity in the pyridine field was not necessarily limited to derivatives of nicotinamide. This meant in effect that the field was wide open and that theoretically, at least, tuberculostatic activity might exist in any pyridine structure.

The potential productivity of the pyridine field as a source of anti-tubercular agents was further pointed up by the discovery in these laboratories and elsewhere of the potent tuberculostatic activity of nicotinaldehyde thiosemicarbazone (III) (2–4), methyl 4-pyridyl ketone thiosemicarbazone (IV) (5, 6), and isonicotinaldehyde thiosemicarbazone (V) (5, 7). These three compounds were much more active than nicotinamide which had been used clinically by Huant (9) and which, up to that time, had been the most active of the pyridine type. Indeed, nicotinaldehyde thiosemicarbazone (III) and isonicotinaldehyde thiosemicarbazone (V) appeared to be at least as active as Tibione (*p*-acetamidobenzaldehyde thiosemicarbazone) itself, and there were some indications that they were actually more active (8).



III IV V

The preparation of isonicotinaldehyde thiosemicarbazone (V) by one of us (H. H. F.) was effected by a modification of the McFadyen-Stevens reaction in which methyl isonicotinate was converted to isonicotinylhydrazine (VI). The atter, in turn, was treated with benzenesulfonyl chloride to give 1-isonicotinyl-2-benzenesulfonylhydrazine (VII) which yielded the desired thiosemicarbazone (V) on alkaline decomposition in the presence of thiosemicarbazide. Since both isonicotinic acid hydrazide (VI) and its benzenesulfonyl derivative (VII) were pyridine carboxylic acid derivatives and therefore were closely related to the structures under investigation, they were submitted to the Chemotherapy Department of the Roche Research Laboratories for testing. The benzenesulfonyl derivative (VII) was shown to be inactive. On the other hand, isonicotinic acid hydrazide (VI) proved to have an *in vivo* anti-tubercular activity which exceeds that of any known substance — whether synthetic or antibiotic.



The notable superiority of isonicotinic acid hydrazide (VI) over streptomycin, for example, is illustrated in Table I where its efficacy, when administered both orally and subcutaneously in mice, is compared to that of streptomycin given subcutaneously. Several points in this comparison are worthy of special mention. In the first instance, unlike streptomycin which is ineffective orally, VI is almost as active orally as it is parenterally. Furthermore, whereas by the subcutaneous route it is approximately 13 times more active than streptomycin in the intravenous type of infection, it is approximately 56 times more active in the intranasal type of infection.

On the basis of earlier experiences in this laboratory with tuberculostats of the pyridine series, it seemed very likely that positional or structural changes in VI would result in diminution or abolition of activity. Nevertheless, it was decided to study in some detail the relationship between structure and activity for derivatives of VI and some related compounds.

The effect of a change in position on the activity was first determined by investigating the *alpha* and *beta* isomers, namely, picolinic acid hydrazide

TABLE I

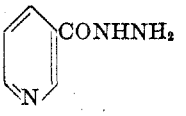
COMPARATIVE ACTIVITIES OF ISONICOTINIC ACID HYDRAZIDE (VI) AND STREPTOMYCIN IN MICE

COMPOUND	ROUTE	LD_{50} (mg./kg.) (a)	INTRAVENOUS TYPE PD_{50} (mg./kg.) (b)	INTRANASAL TYPE PD_{50} (mg./g.) (c)	THERAPEUTIC RATIO	
					a/b	a/c
Streptomycin	subc.	970	25	100	38.8	9.3
Isonicotinic acid hydrazide	subc.	203	1.86	1.77	109	115
	per os	203	4.6	6.2	44.1	32.7

NOTE: PD_{50} = dose protecting 50% of mice. LD_{50} = dose killing 50% mice.

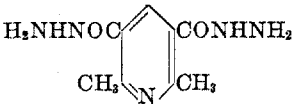
(VIII) and nicotinic acid hydrazide (IX) respectively. Compound VIII was found to be active but very toxic, and IX was found to be inactive.

VIII



IX

The addition of another carboxylic acid hydrazide group in the *beta* position giving of 2,6-dimethyldinicotinic acid hydrazide (X) also produced an inactive

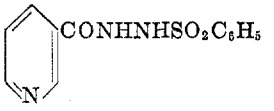


X

compound. The *gamma* position was probably, therefore, the position of choice.

Upon treatment of compounds VIII and IX with benzenesulfonyl chloride, 1-picolinyl-2-benzenesulfonylhydrazine (XI) and 1-nicotinyl-2-benzenesulfonylhydrazine (XII) were obtained respectively. Both compounds were without tuberculostatic activity. It would appear likely, therefore, that aryl sulfonation of the terminal nitrogen was incompatible with activity.

XI

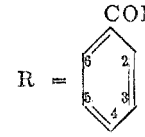


XII

The effect of substituting a benzene nucleus for the pyridine ring was studied by preparing a series of benzoic acid hydrazides with substituents in the *ortho*, *beta*, and *para* positions. The compounds thus prepared are listed in Table II.

TABLE II

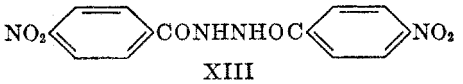
SUBSTITUTED BENZOIC ACID HYDRAZIDES



NUCLEUS	SUBSTITUENT					M.P., °C.	REF.
	2	3	4	5	6		
R	—	—	—	—	—	111–112.5	(10)
R	—	—	NH_2	—	—	219–222	(11)
R	OH	—	—	—	—	148–150	(12)
R	—	—	OH	—	—	262 (dec.)	(12)
R	—	NO_2	—	—	—	153–154	(13)
R	—	—	NO_2	—	—	211–212	(13)
R	—	—	$NHCOCH_3$	—	—	288–289	E
R	OH	—	—	Br	—	217–218	E
R	OH	—	—	OH	—	209–210	E
R	OCH_3	—	—	—	—	78–80	E
R	NH_2	—	—	—	—	121–122	(14)

NOTE: E = see Experimental.

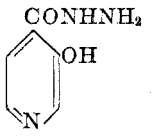
In addition to the monobenzoyl derivatives, the dibenzoyl derivative, 1,2-bis(*p*-nitrobenzoyl)hydrazine XIII was prepared.



XIII

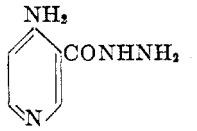
All of the compounds of the benzenoid series were inactive.

In the light of this finding, it was decided to limit the changes to the addition of substituents to the pyridine nucleus and to the hydrazine moiety. Accordingly, 3-hydroxyisonicotinic acid hydrazide (XIV) was prepared from the corresponding methyl ester with hydrazine hydrate. The compound showed no activity.

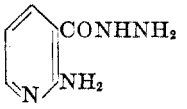


XIV

Similarly, the placement of amino groups in the 4 or 2 positions to give 4-aminonicotinic acid hydrazide (XV) and 2-aminonicotinic acid hydrazide (XVI) was equally unproductive of anti-tubercular activity.



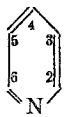
XV



XVI

TABLE III

Pyridine Carboxylic Acid Hydrazides

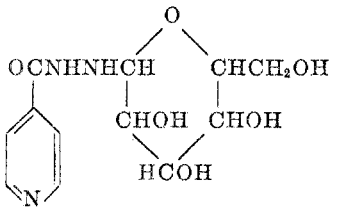
R = 

No.	Nucleus	Substituent					M.p., °C.	Ref.	Activity
		2	3	4	5	6			
VI	R	—	—	$CONHNH_2$	—	—	168.5–170.5	(15)	+
VII	R	—	—	$CONHNHSO_2\phi$	—	—	190	(16)	0
VIII	R	$CONHNH_2$	—	—	—	—	97.5–100	(15)	+
IX	R	—	$CONHNH_2$	—	—	—	159–161	(15)	0
X	R	CH_3	$CONHNH_2$	—	$CONHNH_2$	CH_3	230.5–232.5	E	0
XI	R	$CONHNHSO_2\phi$	—	—	—	—	201–203	(16)	0
XII	R	—	$CONHNHSO_2\phi$	—	—	—	183.5–185.5	(16)	0
XIV	R	—	HO	$CONHNH_2$	—	—	320	E	0
XV	R	—	$CONHNH_2$	NH_2	—	—	206–208	E	0
XVI	R	NH_2	$CONHNH_2$	—	—	—	186.5–187.5	E	0
XVII	R	—	—	CONHNHOCPy	—	—	263–265	(17)	+
XVIII	R	—	—	CONHNH-glucosyl	—	—	160 (dec.)	E	+

Note: E = see Experimental; Py = 4-pyridyl; ϕ = phenyl.

To determine the effect of substitution on the hydrazine moiety, two compounds were prepared. The first of these, 1,2-diisonicotinylhydrazine (XVII), was prepared by reacting isonicotinyl chloride with hydrazine hydrate. The other compound, 1-isonicotinyl-2-D-glucosylhydrazine XVIII, was prepared by reacting isonicotinic acid hydrazine (VI) with glucose.

XVII



XVIII

Both compounds retained a high order of activity, suggesting the possibility that one or both terminal hydrogens on the hydrazide group could be replaced without abolishing the activity. The glucosyl derivative (XVIII) was particularly active and was much less toxic in mice than the parent substance.

The pyridine carboxylic acid hydrazides prepared in this study are listed in Table III. 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.

All melting points in Tables II and III are corrected.

The preparation of those compounds which have not previously appeared in the literature is described in the Experimental section.

Acknowledgment. The authors are indebted to Dr. A. Steyermark and his staff for the microanalyses and to Drs. Schnitzer and Grunberg and the staff of the Chemotherapy Laboratory for testing the compounds.

EXPERIMENTAL

All melting points are corrected.

p-Acetamidobenzoic acid hydrazide. A mixture of 30 g. of ethyl *p*-acetamidobenzoate and 25 cc. of hydrazine hydrate (85%) was heated for ½ hour on a steam-bath and then under reflux for ½ hour. The product was recrystallized from dilute methanol. Small white granular crystals; insoluble in water, methanol, ethanol, and benzene; somewhat more soluble in dilute alcohols; m.p. 288–289°.

Anal. Calc'd for $C_9H_{11}N_3O_2$: C, 56.0; H, 5.7.
Found: C, 56.1; H, 5.6.

2-Hydroxy-5-bromobenzoic acid hydrazide. A mixture of 30 g. of methyl 2-hydroxy-5-bromobenzoate and 40 cc. of hydrazine hydrate (85%) was heated on a steam-bath for 2 hours, at the end of which time the solid ester had gone into solution. The excess hydrazine hydrate was then removed under a vacuum, and the orange-colored residue was recrystallized from 80% 2-propanol. Yield 24 g.; white needles; insoluble in water, methanol, ethanol, and benzene; soluble in hot dilute alcohol; m.p. 217–218°.

Anal. Calc'd for $C_7H_7BrN_2O_2$: C, 36.4; H, 3.1.
Found: C, 36.7; H, 3.2.

Gentisic acid hydrazide. A mixture of 35 g. of methyl gentisate and 18 cc. of hydrazine hydrate (85%) was heated on a steam-bath. The mixture at first liquified and then solidified. The solid product was recrystallized from water. Yield 27 g. of colorless needles; m.p. 209–210°.

Anal. Calc'd for $C_7H_8N_2O_3$: C, 50.0; H, 4.8.
Found: C, 50.0; H, 4.5.

2-Methoxybenzoic acid hydrazine. A mixture of 30 g. of methyl 2-methoxybenzoate and 20 cc. of hydrazine hydrate (85%) was refluxed for 2 hours. The excess of hydrazine hydrate was then removed under a vacuum; the residue solidified on cooling, yield 27 g. Upon recrystallization from toluene or "Skellysolve B", the product was obtained in the form of white crystals which melted at 78–80°; very soluble in water, methanol, ethanol, and 2-propanol; moderately soluble in benzene and "Skellysolve B".

Anal. Calc'd for $C_8H_{10}N_2O_3$: C, 57.8; H, 6.0.
Found: C, 58.0; H, 6.3.

2,6-Dimethyldinicotinic acid dihydrazide. A mixture of 25.1 g. of diethyl 2,6-dimethyldinicotinate and 18 cc. of hydrazine hydrate (85%) was refluxed for 2–3 hours or until the two liquid phases changed to a solid and a liquid phase. The excess hydrazine hydrate was then removed under a vacuum, and the residue was recrystallized from dilute 2-propanol. Colorless needles; very soluble in water; slightly soluble in methanol and ethanol; very slightly soluble in 2-propanol; m.p. 230.5–232.5°.

Anal. Calc'd for $C_9H_{13}N_5O_2$: C, 48.5; H, 5.8.
Found: C, 48.1; H, 5.6.

3-Hydroxyisonicotinic acid hydrazide. A mixture of 10 g. of methyl 3-hydroxyisonicotinate and 6 cc. of hydrazine hydrate (85%) was heated on a steam-bath. The ester dissolved at first and then a precipitate appeared. The mixture was dissolved in hot dilute hydrochloric acid, a small quantity of impurity was filtered off, and the filtrate was adjusted to *p*H 6 to give a precipitate of the product. Recrystallization from dilute 2-propanol gave orange-yellow flakes; m.p. >320°; soluble in dilute acid, ammonium hydroxide, hot ethanol, and hot water; insoluble in cold water, alcohol, and benzene.

Anal. Calc'd for $C_6H_7N_3O_2$: C, 47.1; H, 4.6.
Found: C, 47.2; H, 4.5.

4-Aminonicotinic acid hydrazide. A mixture of 20 g. of methyl 4-aminonicotinate and 40 cc. of hydrazine hydrate (85%) was heated to solution on a steam-bath. The excess of hydrazine hydrate was removed under a vacuum, and the residue was recrystallized from water; yield, 19 g. of long white needles, m.p. 206–208°.

Anal. Calc'd for $C_6H_8N_4O$: C, 47.4; H, 5.3.
Found: C, 47.8; H, 5.6.

2-Aminonicotinic acid hydrazide. A mixture of 25 g. of ethyl 2-aminonicotinate and 35 cc. of hydrazine hydrate (85%) was heated on a steam-bath with occasional shaking until the mixture became a homogeneous solution and then solidified. The product was obtained in quantitative yield. It formed white hairlike crystals from 2-propanol containing a little water; m.p. 186.5–187.5°; very soluble in water; soluble in dilute alcohols; slightly soluble in 2-propanol.

Anal. Calc'd for $C_6H_8N_4O_2$: N, 36.8. Found: N, 37.0.

*1-Isonicotinyl-2-*D*-glucosylhydrazine.* A mixture of 137 g. (1 mole) of isonicotinylhydrazine, 180 g. (1 mole) of anhydrous D-glucose, and 1000 cc. of methanol was refluxed until solution was complete. The reaction mixture was filtered if necessary and then cooled to yield a precipitate of 1-isonicotinyl-2-D-glucosylhydrazine. The precipitate was filtered off, and the filtrate on evaporation to dryness gave the rest of the product in quantitative yield. The compound occurred in two crystalline forms: (a) fine white needles and (b) hard colorless granules. It decomposed at 160° with previous softening and darkening; very soluble in water; soluble in methanol; insoluble in most of the other organic solvents.

Anal. Calc'd for $C_{12}H_{17}N_3O_6$: C, 48.2; H, 5.7.
Found: C, 48.0; H, 5.5.

CONCLUSION

With the discovery that isonicotinic acid hydrazide (VI) is a very potent agent for the treatment of mice infected with *M. tuberculosis*, a whole new field has been opened for investigation and another class of compounds added to the

few chemical types which have shown tuberculostatic activity. This class may be broadly termed "hydrazine derivatives" or, more particularly, "pyridine carboxylic acid hydrazides". It is at present much too early to delimit the field, but preliminary indications are that a benzenoid central nucleus is incompatible with activity and that aryl sulfonyl groups on the terminal nitrogen atom of the hydrazine moiety are also detrimental. It also seems probable that the *gamma* position on the pyridine ring is the position of choice — at least insofar as the carboxylic acid hydrazide derivatives are concerned. Three of the four active compounds described in this study, namely, isonicotinic acid hydrazide (VI), 1,2-diisonicotinic acid hydrazide (XVII), and 1-isonicotinyl-2-D-glucosylhydrazine (XVIII) have carboxylic acid hydrazide groups in the *gamma* position. The single exception, picolinic acid hydrazide (VIII), is very active but very toxic.

SUMMARY

Isonicotinic acid hydrazide has been discovered to be the most powerful synthetic tuberculostat known to date. Two of its derivatives, namely, 1,2-diisonicotinic acid hydrazide and 1-isonicotinyl-2-D-glucosylhydrazine and one of its isomers, namely, picolinic acid hydrazide have also shown very marked — if somewhat diminished — activity.

None of the twelve benzoic acid hydrazide derivatives in this report were active.

NUTLEY, N. J.

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