[CONTRIBUTION NO. 242 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE INC.]

SYNTHETIC TUBERCULOSTATS. III. ISONICOTINALDEHYDE THIOSEMICARBAZONE AND SOME RELATED COMPOUNDS

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During the course of a search for chemotherapeutic agents to be used in the treatment of tuberculosis, a considerable number of pyridine carboxylic acid derivatives were synthesized and tested (1, 2). Of these, only two compounds were found to have distinct activity, although in both instances the activity was less than that of nicotinamide itself. The two compounds were 3-aminoisonicotinic acid and its methyl ester. Unfortunately, it appeared that a high degree of specificity existed between these structures and their tuberculostatic activity so that any positional and structural deviation seemed to result in a loss of the anti-tubercular effect.

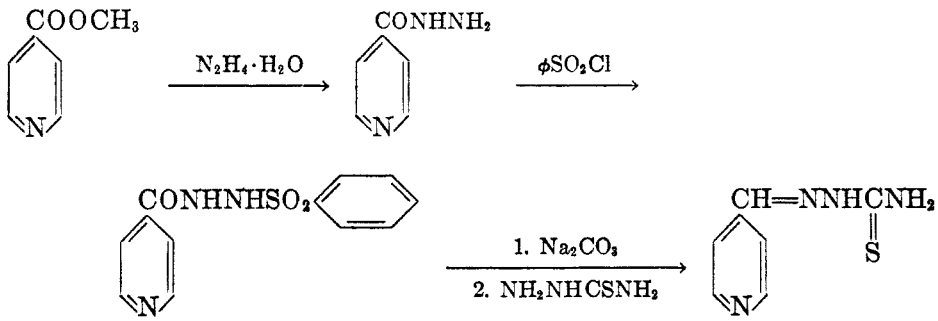
With the advent of *p*-acetamidobenzaldehyde thiosemicarbazone (Tibione) (3, 4) as a new and reputedly clinically effective tuberculostat (5, 6, 7), another line of endeavor became available to workers in the field, and some closely related thiosemicarbazones were made and tested (8, 9), although to date most of the published reports have been confined to Tibione itself. In the light of this work it was decided to prepare the pyridine analog of Tibione and investigate its activity.

In order to prepare isonicotinaldehyde thiosemicarbazone, it was decided to synthesize isonicotinaldehyde and condense it with thiosemicarbazide. A search through the literature, however, quickly revealed that isonicotinaldehyde was not easily obtained. In fact, literature references to isonicotinaldehyde were notable for their absence, and it was only as recently as 1944 that the compound was first successfully prepared and characterized by Wibaut, Kooyman, and Boer (11). The latter workers prepared the aldehyde from *gamma*-picoline by the ozonolysis method of Harries and Lénárt (12) and reported that it was very sensitive to oxidation. In this laboratory the first attempts to synthesize the aldehyde were predicated upon the assumption that *gamma*-picoline could be oxidized to the aldehyde with SeO_2. The assumption proved to be unwarranted, and the only products obtained, despite considerable variation in conditions, were isonicotinic acid and what appeared to be traces of the aldehyde. The next line of approach involved the well-known Stephen's reaction (13) for synthesizing aldehydes from nitriles. A modification of this reaction had been used successfully in these laboratories to prepare nicotinaldehyde thiosemicarbazone (10), but this too failed when applied to *gamma*-cyanopyridine. Recourse was therefore taken to the McFadyen and Stevens' reaction (14), even though Niemann, Lewis, and Hays (15) reported that this latter reaction could be used to make nicotinaldehyde and picolinaldehyde but not isonicotinaldehyde from the corresponding pyridine carboxylic acids. Accordingly, methyl isonicotinate was treated with hydrazine hydrate to give isonicotinic acid hydrazide which gave 1-iso-

nicotinyl-2-benzenesulfonylhydrazine on treatment with benzenesulfonyl chloride. In keeping with the findings of Niemann, Lewis, and Hays, decomposition of the benzenesulfonyl derivative with sodium carbonate in glycerine failed to give identifiable quantities of the aldehyde. It is interesting to note that Niemann and his co-workers explained the differential behavior of the three isomeric pyridine monocarboxylic acids in the following terms:

"McFadyen and Stevens found that *p*-nitrobenzoic acid could not be converted into *p*-nitrobenzaldehyde by their procedure, although *m*-nitrobenzaldehyde was readily obtained from *m*-nitrobenzoic acid. Our observation of the difference in the behavior of nicotinic acid and isonicotinic acid in the McFadyen-Stevens' reaction provides still another example of the parallel behavior of derivatives of pyridine and nitrobenzene. The conversion of picolinic acid to picolinaldehyde by the McFadyen-Stevens' reaction cannot be taken as an exception to the above generalization, because with picolinic benzenesulfonhydrazide there is an excellent possibility that an intramolecular hydrogen bond is formed between the pyridine nitrogen atom and one of the nitrogen atoms present in the side chain, thereby causing a decided structural modification which would preclude any direct comparison of the behavior of the *alpha* and *gamma* compounds."

Despite these overwhelmingly adverse theoretical considerations, it appeared quite likely that the failure was simply due to the rapid destruction of the unstable aldehyde. On this conceptual basis, it was decided to decompose 1-isonicotinyl-2-benzenesulfonylhydrazine in the presence of thiosemicarbazide in the expectation that the thiosemicarbazide would serve to tie up and stabilize the aldehyde as soon as it was formed. The concept was apparently well-founded, since the desired isonicotinaldehyde thiosemicarbazone (I) was readily obtained.

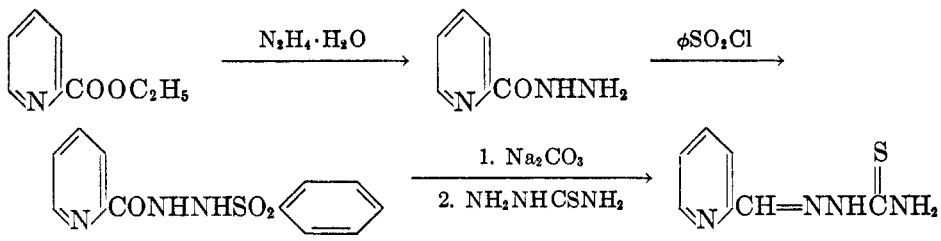


I

For further confirmation of the idea that the failure to obtain isonicotinaldehyde by the McFadyen-Stevens' method was due to its instability, the reaction was run so that the addition of thiosemicarbazide to the reaction mixture was delayed until the alkaline decomposition of the benzenesulfonyl derivative was complete. Under these conditions none of the desired thiosemicarbazone was obtained.

Picolinaldehyde thiosemicarbazone (II) was prepared from 1-picolinyl-2-benzenesulfonylhydrazine in the same way.

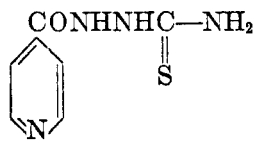
Like its *beta* isomer (10, 16, 17), isonicotinaldehyde thiosemicarbazone (I)



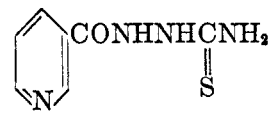
II

proved to be extremely active in tuberculosis infections of mice, whereas picolinaldehyde thiosemicarbazone (II) was shown to be inactive (18).

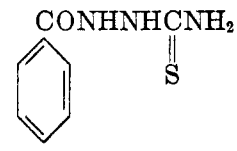
In view of the activity of the *beta* and *gamma* isomers, it seemed desirable to prepare some closely related compounds in the hope of discovering additional structures with tuberculostatic activity. Accordingly, the aldehydo function was first replaced by an acyl grouping, and several thiosemicarbazides were prepared, namely, isonicotinic acid thiosemicarbazide (III), nicotinic acid thiosemicarbazide (IV), benzoic acid thiosemicarbazide (V), and *p*-nitrobenzoic acid thiosemicarbazide (VI). Incidentally, it may be noted in passing that the hydrochloride of compound (IV) was independently prepared by Gardner (10). The preparation of these compounds was effected by condensing thiosemicarbazide with the appropriate acid chloride. None of these compounds was significantly active.



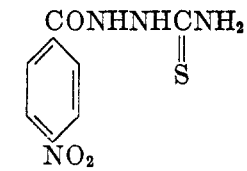
III



IV



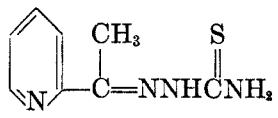
V



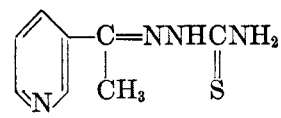
VI

In addition, in order to determine the effect of a keto group on the activity, the method of Kolloff and Hunter (19) and Burrus and Powell (20) was used to prepare the three methyl pyridyl ketones, ethyl 4-pyridyl ketone, and propyl 4-pyridyl ketone, which were then readily condensed with thiosemicarbazide to give methyl 2-pyridyl ketone thiosemicarbazone (VII), methyl 3-pyridyl ketone thiosemicarbazone (VIII), methyl 4-pyridyl ketone thiosemicarbazone (IX), ethyl 4-pyridyl ketone thiosemicarbazone (X), and propyl 4-pyridyl ketone thiosemicarbazone (XI). The methyl 4-pyridyl ketone derivative (IX) proved to be strongly tuberculostatic.[1]

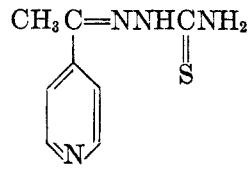
[1] Subsequent to the presentation of this paper at the XIIth International Congress of Pure and Applied Chemistry in September 1951, compounds VII, VIII, and IX were reported by Anderson, Duca, and Scudi [*J. Am. Chem. Soc.*, **73**, 4967 (1951)].



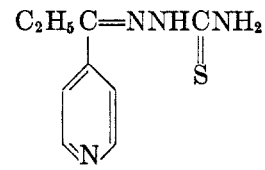
VII



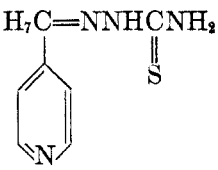
VIII



IX

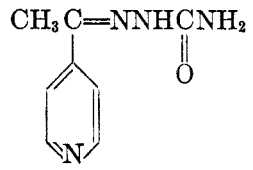


X

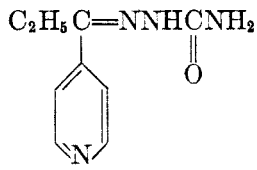


XI

Replacement of the sulfur atom in compounds (IX) and (X) with oxygen to give methyl 4-pyridyl ketone semicarbazone (XII) and ethyl 4-pyridyl ketone semicarbazone (XIII) also resulted in inactive compounds.



XII



XIII

Of the compounds prepared in the present study, therefore, I and IX possess very marked activity against the tuberculosis infection in mice. In intravenous

TABLE I

TUBERCULOSTATIC EFFECT OF THIOSEMICARBAZONES ON THE INTRAVENOUS AND INTRANASAL INFECTIONS OF MICE

COMPOUND	50% CURATIVE DOSE IN MG./KG. OF BODY WEIGHT	
	Intravenous	Intranasal
Tibione	66	899
Isonicotinaldehyde thiosemicarbazone	44	126
Methyl 4-pyridyl ketone thiosemicarbazone	64	>100

infections both compounds appear to have at least the same order of activity as Tibione. As regards the more resistant intranasal infection (18), isonicotinaldehyde thiosemicarbazone (I) seems considerably more active than Tibione, whereas with methyl 4-pyridyl ketone thiosemicarbazone (IX) no definitive figure could be obtained, since its active dose exceeded its tolerated dose. It is interesting to note that both of these compounds as well as 3-aminoisonicotinic acid and its methyl ester (1) all possess substituents in the *gamma*-position.

The activities of compounds I and IX relative to that of Tibione in intravenous and intranasal infections of mice are given in Table I.

Acknowledgment. The author wishes to thank Dr. R. J. Schnitzer, Dr. E. Grunberg, and the staff of the Chemotherapy Deaprtment for their close cooperation in evaluating the compounds. The author also wishes to thank Dr. A. Steyermark and his staff for the microanalyses.

EXPERIMENTAL

All melting points are corrected.

1-Isonicotinyl-2-benzenesulfonylhydrazine. A suspension of 196 g. of isonicotinic acid hydrazide in 1240 cc. of pyridine was treated with 260 g. of benzenesulfonyl chloride according to the method of Niemann, Lewis, and Hays (15). The white, crystalline product weighed 285 g., dec. 190° [Niemann, Lewis, and Hays m.p. 193–194° (15)].

Isonicotinaldehyde thiosemicarbazone. A mixture of 25 g. of 1-isonicotinyl-2-benzenesulfonylhydrazine, 24 g. of anhydrous sodium carbonate, 9 g. of thiosemicarbazide, and 100 cc. of glycerine in a large flask was heated to 160° and maintained at that temperature for 2 minutes. During the heating marked foaming began at about 120° and continued as the temperature mounted to 160°. At the end of 2 minutes at 160°, 100 cc. of water was added and the reaction mixture was cooled. On standing, 5 g. (31%) of pure isonicotinaldehyde thiosemicarbazone precipitated out, dec. 219–220°. The product occurs in two crystalline forms: fine, white needles and tan granules. The granules are the more stable variety and are formed by treating the needles with methanol at room temperature or by heating them with water.

Anal. Calc'd for $C_7H_8N_4S$: C, 46.7; H, 4.5; N, 31.1.

Found: C, 46.4; H, 4.5; N, 30.5.

The *monohydrochloride* was obtained by dissolving a portion of the base in hot, dilute hydrochloric acid. On cooling, deep yellow crystals were obtained, dec. 269°.

Anal. Calc'd for $C_7H_8N_4S \cdot HCl$: C, 38.8; H, 4.2.

Found: C, 38.7; H, 3.8.

The *ethanesulfonic acid salt* was prepared by suspending 20 g. of the free base in 75 cc. of water and adding 15 g. of ethanesulfonic acid to the mixture. The free base dissolved to give a clear solution which deposited bright yellow prisms of the salt on cooling and standing, dec. 208°. Addition of acetone to the aqueous filtrate after removal of the salt resulted in a further precipitation of the product.

Anal. Calc'd for $C_7H_8N_4S \cdot C_2H_5SO_3H \cdot H_2O$: C, 34.8; H, 5.2.

Found: C, 35.1; H, 4.6.

1-Picolinyl-2-benzenesulfonylhydrazine. Picolinic acid hydrazide (93.5 g.) in 650 cc. of pyridine was treated with 130 g. (95 cc.) of benzenesulfonyl chloride in accordance with the method of Niemann, Lewis, and Hays (15) to yield 182 g. of the product; m.p. 201–203° (Ref. 15, m.p. 202–203.5°).

Picolinaldehyde thiosemicarbazone. A mixture of 25 g. of 1-picolinyl-2-benzenesulfonylhydrazine, 15 g. of anhydrous sodium carbonate, 9.1 g. of thiosemicarbazide, and 80 cc. of ethylene glycol was heated to 160° and kept at that temperature until the evolution of gas practically ceased (about 30 minutes). To the reaction mixture was then added 180 cc. of water, and the resulting solution was quickly filtered. On cooling and standing, yellow crystals of picolinaldehyde thiosemicarbazone (7.5 g.) precipitated out. Long, yellow needles from dilute methanol, dec. 204–205°.

Anal. Calc'd for $C_7H_8N_4S$: C, 46.7; H, 4.5.

Found: C, 46.4; H, 4.1.

The *monohydrochloride* was prepared by dissolving the base in hot, dilute hydrochloric acid. On cooling the acid solution, lustrous, golden yellow needles of the hydrochloride precipitated out, dec. 230°.

Isonicotinic acid thiosemicarbazide. A solution of 21 g. of isonicotinic acid chloride in 150 cc. of dry pyridine was added dropwise with stirring to a suspension of 13.6 g. of thiosemicarbazide in 100 cc. of dry pyridine. When addition was complete, the mixture was

refluxed for 30 minutes. About one-half of the pyridine was then removed under a vacuum, and the residue was diluted with 500 cc. of water. On cooling, the crude isonicotinic acid thiosemicarbazide precipitated out and was purified by reprecipitation from dilute ammonium hydroxide with dilute acetic acid. White to yellow needles, dec. 279–280°.

Anal. Calc'd for $C_7H_8N_4OS$: C, 42.8; H, 4.1.
Found: C, 43.1; H, 4.0.

Nicotinic acid thiosemicarbazide. A solution of 25 g. of nicotinic acid chloride in 165 cc. of dry pyridine was added portionwise with stirring to a suspension of 15.5 g. of thiosemicarbazide in 135 cc. of pyridine. The reaction mixture was refluxed for 30 minutes and was then worked up as described above to give nicotinic acid thiosemicarbazide. Small, white crystals from dilute methanol; m.p. 179.5°.

Anal. Calc'd for $C_7H_8N_4OS$: C, 42.8; H, 4.1.
Found: C, 42.3; H, 4.7.

Benzoic acid thiosemicarbazide. This compound was previously described by Gaertner (21) who reported that it melted at 200°. Since the product obtained in this study melted 17° lower, its synthesis and analysis are given.

A solution of 25 g. of benzoyl chloride in 165 cc. of dry pyridine was added portionwise with stirring to a suspension of 15.5 g. of thiosemicarbazide in 135 cc. of pyridine. The reaction mixture was refluxed for 40 minutes, the pyridine was then removed under a vacuum, and 300 cc. of water was added to the residue to give a precipitate of the product. White flakes from water; m.p. 183–183.5° [Gaertner, dec. 200° (21)].

Anal. Calc'd for $C_8H_9N_3OS$: C, 49.3; H, 4.6.
Found: C, 49.7; H, 4.1.

p-Nitrobenzoic acid thiosemicarbazide. To 14.7 g. of thiosemicarbazide dissolved in 200 cc. of boiling pyridine was added dropwise 30 g. of *p*-nitrobenzoyl chloride in 250 cc. of pyridine. The addition took about 30 minutes, and the reaction mixture was refluxed for 30 minutes longer. On cooling, crystals of *p*-nitrobenzoic acid thiosemicarbazide precipitated out. They were purified by reprecipitation from dilute ammonium hydroxide with dilute hydrochloric acid and by recrystallization from dilute ethanol, dec. 206°. The product exists in two crystalline forms: white needles and yellow prisms. On analysis, the white needles corresponded to the hemihydrate and the yellow prisms to the hydrate.

Anal. Calc'd for $C_8H_8N_4O_3S \cdot 0.5\ H_2O$: C, 38.6; H, 3.6.
Found (white needles): C, 38.8; H, 4.2.

Anal. Calc'd for $C_8H_8N_4O_3S \cdot H_2O$: C, 37.2; H, 3.9.
Found (yellow prisms): C, 37.6; H, 4.2.

Methyl 4-pyridyl ketone thiosemicarbazone hydrochloride. Methyl 4-pyridyl ketone (20 g.), prepared according to the method of Burrus and Powell (20), was mixed with 15 g. of thiosemicarbazide, and the mixture was heated at 100–130°. After a short time, the mixture solidified, and the solid was recrystallized from hot, dilute hydrochloric acid. The methyl 4-pyridyl ketone thiosemicarbazone hydrochloride precipitated as bright yellow needles (26 g.), dec. 247–248°.

Anal. Calc'd for $C_8H_{10}N_4S \cdot HCl$: C, 41.7; H, 4.8.
Found: C, 42.1; H, 4.8.

A portion of the solid reaction product was purified by solution in hot, dilute sodium hydroxide and reprecipitation with dilute acetic acid at *p*H 6–7 to give the pure, *free base.* White microcrystals, dec. 230°.

Methyl 3-pyridyl ketone thiosemicarbazone. Methyl 3-pyridyl ketone (27 g.), prepared according to the method of Burrus and Powell (20), was mixed with 20.5 g. of thiosemicarbazide and heated until the inside temperature was 105°, at which point the reaction mixture began to foam and then solidified. The solid methyl 3-pyridyl ketone thiosemicarbazone was purified by reprecipitation from hot, dilute hydrochloric acid with dilute ammonium hydroxide to give near white needles, dec. 217°.

Anal. Calc'd for $C_8H_{10}N_4S$: C, 49.5; H, 5.2.
Found: C, 49.3; H, 5.1.

Methyl 2-pyridyl ketone thiosemicarbazone. Methyl 2-pyridyl ketone (30 g.), prepared according to the method of Burrus and Powell (20), was heated to 135° with 22.8 g. of thiosemicarbazide. The mixture frothed vigorously and then solidified. The solid product was recrystallized from ethanol. Yellow crystals; m.p. 160.5–161°.

Anal. Calc'd for $C_8H_{10}N_4S$: C, 49.5; H, 5.2.
Found: C, 49.2; H, 5.5.

Ethyl 4-pyridyl ketone thiosemicarbazone. Ethyl 4-pyridyl ketone (12 g.) (20), 12 g. of thiosemicarbazide, and 75 cc. of ethylene glycol were mixed and heated for 20 minutes at 140–150°. To the cooled mixture was added about 600 cc. of water, upon which the product precipitated. Recrystallization from dilute ethanol gave white plates; m.p. (dec.) 189.5–190.5°.

Anal. Calc'd for $C_9H_{12}N_4S$: C, 52.0; H, 5.8.
Found: C, 51.9; H, 5.9.

Propyl 4-pyridyl ketone thiosemicarbazone. A mixture of 17 g. of propyl 4-pyridyl ketone (20), 12 g. of thiosemicarbazide, and 50 cc. of ethylene glycol was heated at 120–125° for 45 minutes. The hot mixture was diluted with about 200 cc. of water and then cooled to give 11.5 g. of pure product. White needles; m.p. 175.5–176.5°.

Anal. Calc'd for $C_{10}H_{14}N_4S$: C, 54.1; H, 6.3.
Found: C, 54.4; H, 6.4.

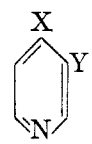
Methyl 4-pyridyl ketone semicarbazone hydrochloride. A mixture of 14 g. of methyl 4-pyridyl ketone, 14 g. of semicarbazide hydrochloride, and 21 g. of sodium acetate in 125 cc. of water was heated to solution and allowed to cool slowly. The precipitated product (20 g.) proved to be the hydrochloride and was obtained in the form of white needles on recrystallization from methanol; m.p. (dec.) 220°.

Anal. Calc'd for $C_8H_{10}N_4O \cdot HCl$: C, 44.8; H, 5.1.
Found: C, 45.0; H, 5.3.

Ethyl 4-pyridyl ketone semicarbazone. A solution of 14 g. of ethyl 4-pyridyl ketone in 20 cc. of ethanol was added to 14 g. of semicarbazide hydrochloride and 21 g. of sodium acetate in 80 cc. of water. The mixture was heated to solution and then for ½ hour longer. It was then concentrated to about 40 cc. and finally cooled to give 11 g. of product. White needles from water; m.p. 196.5–198.5°.

Anal. Calc'd for $C_9H_{12}N_4O$: C, 56.2; H, 6.3.
Found: C, 55.7; H, 6.2.

Conclusion. The results of this and other studies done in this field suggest that the pyridine nucleus offers a promising starting point for synthetic tuberculostats. So far the following pyridine structures have shown tuberculostatic activity:



	X	Y
1.	H	$CONH_2$
2.	COOH	NH_2
3.	$COOCH_3$	NH_2

	X	Y
4.	$CH{=}NNHC(=S)NH_2$	H
5.	H	$CH{=}NNHC(=S)NH_2$
6.	$C(CH_3){=}NNHC(=S)NH_2$	H

Though admittedly it is much too early to draw hard and fast rules relating position and activity, it would appear that the *beta* and *gamma* positions are the positions of choice on the pyridine ring for tuberculostatic activity.

SUMMARY

Two new and very active tuberculostats, namely, isonicotinaldehyde thiosemicarbazone and methyl 4-pyridyl ketone thiosemicarbazone have been synthesized. Both appear to be at least as active as Tibione in intravenous mouse infections. In intranasal infections isonicotinaldehyde thiosemicarbazone is apparently considerably more active than Tibione, whereas the activity of methyl 4-pyridyl ketone thiosemicarbazone could not be determined because its active dose exceeded its tolerated dose. All of the other thiosemicarbazones, thiosemicarbazides, and semicarbazones synthesized in this study were inactive.

NUTLEY 10, N. J.

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