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THE SYNTHESIS OF COMPOUNDS FOR THE CHEMOTHERAPY OF TUBERCULOSIS. I. HETEROCYCLIC THIOSEMICARBAZIDE DERIVATIVES

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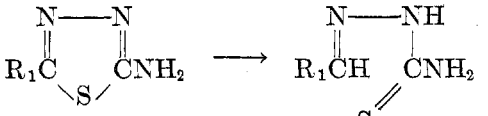
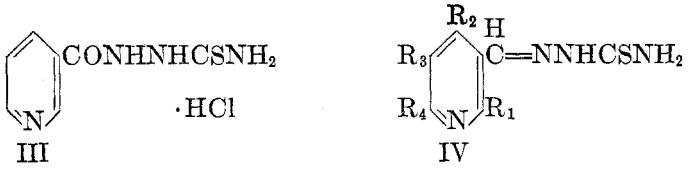
The modest activity of 2-(*p*-aminobenzenesulfonylamino)thiadiazole derivatives against *in vivo* tuberculosis infections as observed by Domagk suggested to Behnisch and his collaborators (1) an examination of the structure of type I.



I II

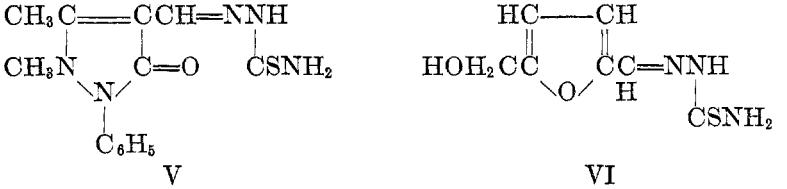
While this type of structure does not appear to have given worthwhile compounds, it was found that structures of type II, which might be considered as open models of type I, displayed activity. The preferred compound, *p*-acetamidobenzaldehyde thiosemicarbazone (II, R = acetaminophenyl), also known as TBI-698 or Conteben (1) in Germany and Tibione in the United States, showed high activity *in vitro* and *in vivo* tests (2). Clinical reports have appeared in France and Germany. Confirmation of activity in animals and man has appeared in England (3) and elsewhere.

Nicotinic acid and nicotinamide have been reported to have *in vivo* antitubercular activity in mice (4). Various derivatives and alterations in the structure of nicotinic acid were shown to decrease the activity (4). On the grounds of the activity of these two types, structures of the type II (R = pyridyl, pyrazolyl, furyl) seemed to be of interest.



III IV

IVa $R_1 = R_2 = R_3 = R_4 = H$
IVb $R_1 = OH, R_2 = CH_2OC_2H_5, R_3 = H, R_4 = CH_3$
IVc $R_1 = H, R_2 = R_4 = CH_3, R_3 = OH$
IVd $R_1 = H, R_2 = CH{=}NNHCSNH_2, R_3 = OH, R_4 = CH_3$



V VI

Nicotinylthiosemicarbazide hydrochloride (III) was prepared as a derivative of nicotinic acid. Nicotinaldehyde thiosemicarbazone (IVa) and its hydrochloride were prepared as derivatives of the aldehyde. Nicotinaldehyde has been prepared by several methods in low yields by various investigators, two of the better methods giving about 35% of the aldehyde in several steps. The McFadyen-Stevens reaction (5), in which nicotinylbenzenesulfonhydrazide was decomposed to the aldehyde (6, 7) and a variation (8) of the Sonn-Müller type reduction (9), in which the product from the reaction of PCl_5 on nicotinylethylamide was reacted with $SnCl_2$ in ether saturated with HCl, gave approximately the same yields (6, 7). Neither method is well suited to larger scale production.

An attempt was therefore made to modify the Stephens reaction (10). In order to avoid ready destruction of the β-pyridyl aldehyde, which may be the cause of poor yields using other methods, the imino complex was hydrolyzed in the presence of thiosemicarbazide. Also, since the Stephens reaction occurs in a heterogeneous system, the replacement of ethyl ether by other ethers was investigated. It was found that diethylene glycol diethyl ether gave a homogeneous system and a much higher yield of the intermediate nicotinaldehyde thiosemicarbazone hydrochloride stannous chloride complex. The use of ethers other than ethyl ether for the Stephens reaction seems to be new and might find larger application. Nicotinaldehyde thiosemicarbazone hydrochloride was obtained from the nicotinaldehyde thiosemicarbazone hydrochloride stannous chloride complex by removal of tin with hydrogen sulfide and purification of the free base.

4-Ethoxymethyl-2-hydroxy-6-methyl-nicotinaldehyde thiosemicarbazone (IVb) was prepared in a similar manner.

Two other 3-pyridine aldehyde thiosemicarbazone derivatives have been prepared. 4,6-Dimethyl-5-hydroxy-nicotinaldehyde thiosemicarbazone (IVc) and 5-hydroxy-6-methylcinchomeronaldehyde bisthiosemicarbazone (IVd) were prepared by modifying the Sommelet reaction. The hexamethylenetetrammonium complex, obtained by refluxing the substituted pyridylmethyl chloride with hexamethylenetetramine in a solvent, was hydrolyzed in the presence of thiosemicarbazide. The yields were low.

Antipyraldehyde thiosemicarbazone (V) and 5-hydroxymethyl-2-furfuraldehyde thiosemicarbazone (VI) were prepared by conventional methods.

CHEMOTHERAPEUTIC RESULTS

All of the thiosemicarbazide derivatives reported here were inactive against tuberculosis in mice with the exception of nicotinaldehyde thiosemicarbazone and its hydrochloride which exhibited very high activity against tuberculosis in mice. The activity was of the order of that of Tibione (11), and since this work was completed, the activity of this compound has been confirmed by Levaditi (12, 13) in mice and guinea pigs. Nicotinaldehyde thiosemicarbazone hydrochloride is soluble in water, and this may be an advantage over Tibione. The high solubility also permits higher blood levels at the same dosage levels. A detailed description of the chemotherapy and pharmacology of nicotinaldehyde thiosemicarbazone will be reported elsewhere.

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EXPERIMENTAL

All melting points are uncorrected.

Nicotinylthiosemicarbazide hydrochloride (III). Nicotinyl chloride hydrochloride (15 g.) was refluxed for 2 hours with thiosemicarbazide (15 g.) in glacial acetic acid. On cooling, a white, crystalline material separated which was recrystallized from acetic acid. The product was soluble in water and glycerine and insoluble in alcohol and ether; m.p. 210–211°; yield 5.5 g. (28%).

Anal. Calc'd for $C_7H_8N_4OS \cdot HCl$: S, 13.8. Found: S, 13.8.

Nicotinaldehyde thiosemicarbazone hydrochloride stannous chloride complex. Under anhydrous reaction conditions, anhydrous stannous chloride (950 g.) was stirred in 3 l. of anhydrous diethylene glycol diethyl ether and dry HCl gas was passed in to saturation, the reaction mixture being held at 45°. Powdered β-cyanopyridine (104 g.) was added with continued stirring and HCl gas passage for 6 hours. Complete solution usually occurred at this time (occasionally when solution was not complete, stirring was continued for 24 hours). The amber solution was dropped into 4 l. of 80° water containing 91 g. of thiosemicarbazide in a period of 30 minutes. The hydrolysis mixture was stirred vigorously and heated at 100° for 2 hours to complete the reaction. The reaction product began to separate while the mixture was still hot and separation was completed by cooling to 10° before filtering; m.p. 214–215°; yield 351 g. (86.5%). On recrystallization from boiling water, a yellow, highly crystalline material was obtained; m.p. 218–219°.

Anal. Calc'd for $C_7H_8N_4S \cdot HCl \cdot SnCl_2$: S, 8.1. Found: S, 8.2.

An investigation into the possibility of changing the solvent and the reaction conditions was made. Ether gave 20% of the complex if 1 mole of $SnCl_2$ was used and up to 50–60% on using 5 moles of $SnCl_2$. The use of 10 moles of $SnCl_2$ did not increase the yield further. A ratio of 5 moles of $SnCl_2$ per mole of β-cyanopyridine was adopted for investigation of other solvent systems. Under these conditions, dioxane gave 21% of the complex; Diethyl Cellosolve (ethylene glycol diethyl ether), 30%; dibutoxytetraglycol 22%; and Diethyl Carbitol (diethylene glycol diethyl ether), 70–90%. Isopropyl ether and *n*-butyl ether failed to give any product. Diethyl Carbitol was adopted for repetitions of the preparation of nicotinaldehyde thiosemicarbazone hydrochloride stannous chloride complex.

Nicotinaldehyde thiosemicarbazone (IVa).[1] Nicotinaldehyde thiosemicarbazone hydrochloride stannous chloride complex (308 g.) was dissolved in 10 l. of boiling water, and hydrogen sulfide was passed in while the solution was being stirred and kept at 90–100°. Complete precipitation of the SnS occurred in about 2½ hours. The precipitated SnS was removed, the filtrate concentrated *in vacuo* to about 3 l., and 300 ml. of conc'd ammonia solution was added (slight excess). Yield, 113 g. (83%) of nicotinaldehyde thiosemicarbazone, m.p. 217°, upon filtration and washing with cold water. A sample was recrystallized several times from boiling water to yield a colorless, crystalline product; m.p. 222–223° (dec.).

Anal. Calc'd for $C_7H_8N_4S$: C, 46.7; H, 4.5; S, 17.8.

Found: C, 46.9; H, 4.6; S, 17.6.

Nicotinaldehyde thiosemicarbazone hydrochloride. Nicotinaldehyde thiosemicarbazone (112 g.) was dissolved in 525 ml. of 12% hot hydrochloric acid solution and filtered hot. Four liters of ethanol was added to the hot solution and the solution cooled to 4°. On filtra

[1] This compound was also prepared independently and by a different method by Drs. R. Schläpfer and H. Spiegelberg in the laboratories of F. Hoffmann-La Roche and Co., Basle, Switzerland.

tion and washing with acetone and then ether, 120 g. (89%) of a yellow, crystalline compound was obtained.

Anal. Calc'd for $C_7H_8N_4S \cdot HCl$: S, 14.7. Found: S, 14.3.

4-Ethoxymethyl-2-hydroxy-6-methyl-nicotinaldehyde thiosemicarbazone (IVb). Anhydrous stannous chloride (189.6 g.) was treated with dry HCl gas in 380 ml. of diethylene glycol diethyl ether until saturated. 4-Ethoxymethyl-2-hydroxy-6-methylnicotinonitrile (38.4 g.) was added and HCl introduction continued for an additional 6 hours. The reaction mixture was hydrolyzed by adding 500 ml. of water containing 18.3 g. of thiosemicarbazide and heating at 100° for 2 hours. On cooling, 59.2 g. of a tin complex separated; m.p. 177–183°. The complex was dissolved in 1500 ml. of boiling water and H_2S passed in to precipitate the SnS. The tin-free filtrate, after removal of SnS, was concentrated and two fractions were obtained, the bulk of the material being in the first (20 g.). The first fraction was dissolved in dilute sodium hydroxide and precipitated with carbon dioxide giving 7 g. which had a trace of ash; the ash was removed by triturating with hot water. A yellow compound (5.4 g.) was obtained which melted above 250°.

Anal. Calc'd for $C_{11}H_{16}N_4O_2S$: S, 11.9. Found: S, 11.8.

3-Chloromethyl-4,6-dimethyl-5-hydroxypyridine hydrochloride. 3-Hydroxymethyl-4,6-dimethyl-5-hydroxypyridine (46 g.) was suspended in 200 ml. of chloroform and heated at 60–70° with 100 ml. of thionyl chloride for 15 hours. The product was filtered, washed with chloroform, and dried at 80° *in vacuo*.

Anal. Calc'd for $C_8H_{10}ClNO \cdot HCl$: Cl, 34.3. Found: Cl, 35.2.

4,6-Dimethyl-5-hydroxy-nicotinaldehyde thiosemicarbazone (IVc). 3-Chloromethyl-4,6-dimethyl-5-hydroxypyridine hydrochloride (41.6 g.) was dissolved in 960 ml. of 60% ethanol. Sodium bicarbonate (16.3 g.) was added, followed by 28 g. of hexamethylenetetramine. The reaction mixture was refluxed for 4 hours at 100–110° and cooled to 50°. Anhydrous sodium acetate (31 g.) and 36 g. of thiosemicarbazide were added and refluxing was continued for an additional 30 minutes. On cooling, 15.5 g. (34%) of crude product separated which was recrystallized from ethanol as a buff-colored compound; m.p. 212–213° (dec.).

Anal. Calc'd for $C_9H_{12}N_4OS$: N, 12.5 (2 N by Kjeldahl). Found: N, 12.0.

5-Hydroxy-6-methylcinchomeronaldehyde bisthiosemicarbazone (IVd). 4-Ethoxymethyl-5-hydroxy-3-hydroxymethyl-6-methylpyridine hydrochloride (21 g.) was suspended in 50 ml. of chloroform and warmed at 40–45° for 4–5 hours with 8.2 ml. of thionyl chloride. The excess $SOCl_2$ was distilled off with the chloroform, and the residue was dissolved in 50 ml. of chloroform. Hexamethylenetetramine (25.2 g.) was added portion-wise during 20 minutes. After refluxing for an additional hour, the reaction mixture was cooled, filtered, and the residue triturated with ether. The complex (36 g.) was hydrolyzed in 330 ml. of 60% ethanol with 12 g. of sodium acetate and 14 g. of thiosemicarbazide under reflux for 30 minutes. About half of the solvent was distilled *in vacuo*, and on cooling, 8.4 g. of product separated. This weighed 3 g. (9%) on crystallization from hot ethanol; m.p. 144–146° (dec.).

Anal. Calc'd for $C_{10}H_{13}N_7OS_2$: S, 20.6; N, 18.0 (4 N by Kjeldahl).
Found: S, 20.0; N, 17.6.

Antipyraldehyde thiosemicarbazone (V). Antipyraldehyde (31 g.) was refluxed with 13.2 g. of thiosemicarbazide in 250 ml. of water containing 5 g. of sodium acetate for 2 hours. On cooling, a yellow compound separated which could be crystallized from hot water, alcohol, or acetone; m.p. 226–227°; yield 37 g. (90%).

Anal. Calc'd for $C_{13}H_{15}N_5OS$: C, 53.9; H, 5.2; S, 11.0.
Found: C, 53.9; H, 5.3; S, 10.6.

5-Hydroxymethyl-2-furfuraldehyde thiosemicarbazone (VI).[2] Thiosemicarbazide (9 g.) was heated in ethanol-water-acetic acid solution with 5-hydroxymethyl-2-furfuraldehyde (13 g.) for 1 hour. On cooling, the product crystallized and was recrystallized from hot ethanol; m.p. 190°; yield 13 g. (76%).

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[2] Prepared by Dr. A. Ziering.

Anal. Calc'd for $C_7H_8N_8O_2S$: C, 42.2; H, 4.5.
Found: C, 42.3; H, 4.5.

SUMMARY

Several heterocyclic derivatives of thiosemicarbazide were prepared. Nicotinaldehyde thiosemicarbazone and its hydrochloride were found to be of the same order of activity as *p*-acetamidobenzaldehyde thiosemicarbazone (Tibione) in tuberculosis in mice.

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