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# THE SYNTHESIS OF COMPOUNDS FOR THE CHEMOTHERAPY OF TUBERCULOSIS. V. SOME TRANSFORMATIONS OF PYRIDYLALDEHYDE THIOSEMICARBAZONES

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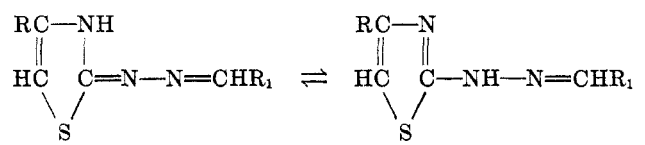
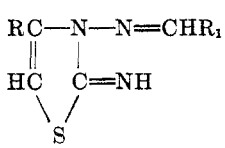
With the finding in these laboratories that nicotinaldehyde thiosemicarbazone hydrochloride (1) had high anti-tubercular activity in mice (2), some modifications of this structure were made along established lines (3, 4). Surprising differences in the biological activity of apparently identical substances made by slightly different techniques which were observed in our Chemotherapy Laboratory have led us to look into the matter more closely.

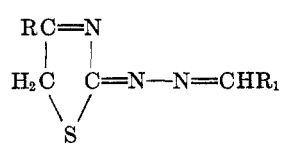
A series of substituted thiazoles was prepared by condensing chloroacetone, chloroacetophenone, and chloroacetal with pyridine aldehyde thiosemicarbazones. The following general structures may be considered for this type of condensation:



I



II



III

Structure I (R = phenyl; $R_1$ = *p*-acetamidophenyl) has been assigned by Wollenberg (4) to the reaction product of *p*-acetamidobenzaldehyde thiosemicarbazone and ω-chloroacetophenone. Structure II (R,$R_1$ = phenyl) has been assigned by Mietzsch (6) to the reaction product of benzaldehyde thiosemicarbazone and ω-chloroacetophenone.

Structures of the type III might also come into consideration for these reaction products.

Two types of condensation conditions were used. In one type, the free base of R—CH=N—NHCSN$H_2$ was condensed in methanol with chloroacetone to give Ia (R = $CH_3$, $R_1$ = 2-pyridyl), Ib (R = $CH_3$, $R_1$ = 3-pyridyl), and Ic (R = $CH_3$, $R_1$ = 4-pyridyl), and with chloroacetophenone to give Id (R = phenyl, $R_1$ = 4-pyridyl). Ia, b, c and d did not immediately evolve ammonia on heating with 50% sodium hydroxide solution and on this basis have been assigned structures corresponding to I.

In the second type of condensation in methanol, nicotinaldehyde thiosemicarbazone hydrochloride was condensed with chloroacetone to give IIa (R =

$CH_3$, $R_1$ = 3-pyridyl); with chloroacetal to give IIb (R = H, $R_1$ = 3-pyridyl), and with chloroacetophenone to give IIc (R = phenyl, $R_1$ = 3-pyridyl). In this type of condensation all of the compounds (II, a, b, c) immediately evolved ammonia on heating with 50% sodium hydroxide and on this basis have been assigned formula II. Heating IIa with 15% NaOH for one hour gave 30% of the available ammonia from the imino group, while Ib gave only a trace of ammonia under the same conditions. All of the thiazole type compounds gave dihydrochlorides with one exception, Id, which was very insoluble in methanol and crystallized out as a monohydrochloride.

In the reaction with the free thiosemicarbazone, condensation occurs with the primary amino group (I), but at low *p*H this group is apparently involved in salt formation and condensation occurs at the secondary amino group (II). Ultraviolet absorption spectra at various *p*H values and infrared spectra of the two isomeric forms (Ib, IIa, R = $CH_3$, $R_1$ = 3-pyridyl) yield no information as to structure. However, the presence of the characteristic absorption for the =NH group in the I.R. at 3.0 $\mu$ in both types eliminates structure III from consideration. Both forms (Ib, IIa) gave two *p*K values near 3 and at 11.5 in addition to the one for the pyridine ring at 4.5–5.

Recent papers on the condensation of thiosemicarbazide hydrochloride and chloroacetone (11) and on thiosemicarbazide base (12) also demonstrate the effects of the condensing medium and support these results.

We are indebted to Drs. E. Grunberg and R. J. Schnitzer of our Chemotherapy Laboratory for the finding that the dihydrochloride of Ib had no activity in tuberculosis in mice and was inactive in the *M. lepraemurium* infection in mice and rats (5). The free base of Ib was also inactive in tuberculosis in mice. The dihydrochloride of IIa has an activity of the order of Promin in the *M. lepraemurium* infection in mice and rats (5) and both the dihydrochloride and the free base have an activity of the order of *p*-aminosalicylic acid in tuberculosis in mice.

Mild oxidation (7, 8) of nicotinaldehyde thiosemicarbazone yielded 2-amino-5-(3-pyridyl)-1,3,4-thiadiazole. This showed no anti-tubercular activity and this class was not further investigated.

Replacement of the sulfur in nicotinaldehyde thiosemicarbazone by the isosteric NH group gave the guanylhydrazone which also had no chemotherapeutic interest. The compound was prepared by the reduction of 3-cyanopyridine according to Stephen and the complex obtained was reacted with aminoguanidine carbonate. This modification of the Stephen reaction (1) was also used to prepare the thiosemicarbazone of nicotinaldehyde methochloride directly and was also found convenient for preparing the known nicotinaldehyde semicarbazone in quantity. Attempts to prepare a nitroguanylhydrazone by the same means gave the guanylhydrazone with loss of the nitro group and nitroguanidine itself did not undergo the reaction.

*Acknowledgment.* We are indebted to Dr. A. Steyermark and his associates for the microanalyses.

## EXPERIMENTAL[1]

*2,3-Dihydro-4-methyl-2-(2-pyridylmethylenehydrazono)thiazole dihydrochloride* (Ia). Picolinaldehyde thiosemicarbazone (5 g.) was heated in 250 ml. of methanol containing 30 ml. of chloroacetone for 8 hours. The excess of chloroacetone and methanol was removed by distillation and the residue was crystallized from hot methanol contaning hydrochloric acid; yield, 6 g.; m.p. 238–240° (dec.), of an orange-colored material.

*Anal.* Calc'd for $C_{10}H_{10}N_4S \cdot 2HCl$: C, 41.3; H, 4.1.
Found: C, 41.5; H, 4.2.

*2-Imino-4-methyl-3-(3-pyridylmethyleneamino)-4-thiazoline dihydrochloride* (IIa). Nicotinaldehyde thiosemicarbazone hydrochloride (122 g.), chloroacetone (110 g.), and methanol (4000 ml.) were mixed to form a suspension with a potential[2] of +385 m.v. in a glass-calomel electrode system. The suspension was heated at reflux with stirring for 23 hours. On cooling, the potential rose to +401 m.v. The solution was evaporated to dryness and the yellow solid was recrystallized from methanol; yield, 108 g.; m.p. 228–230° (dec.).

*Anal.* Calc'd for $C_{10}H_{10}N_4S \cdot 2HCl \cdot H_2O$: C, 39.0; H, 4.5; N, 18.1; S, 10.4; $H_2O$, 5.8.
Found: C, 39.9; H, 4.5; N, 17.5; S, 10.5; $H_2O$, 5.6.

The *free base* was obtained by treating a water solution of the dihydrochloride with ammonia. Recrystallization from methanol gave a pale yellow solid; m.p. 210–212° (dec.).

*Anal.* Calc'd for $C_{10}H_{10}N_4S$: C, 55.0; H, 4.6.
Found: C, 54.8; H, 4.6.

*2,3-Dihydro-4-methyl-2-(3-pyridylmethylenehydrazono)thiazole dihydrochloride* (Ib). Nicotinaldehyde thiosemicarbazone (114 g.), chloroacetone (75 g.), and methanol (4000 ml.) were mixed to give a suspension with a potential of +147 m.v. The suspension was heated at reflux for 5 hours and an additional 75 g. of chloracetone was added and refluxing was continued for 18 hours. After cooling, the suspension had a potential of +295 m.v. The pale yellow recovered product was dissolved in hot methanolic hydrogen chloride solution, decolorized with carbon, and cooled. Yield of recrystallization product, 110 g.; m.p. 225–226° (dec.).

*Anal.* Calc'd for $C_{10}H_{10}N_4S \cdot 2HCl$: C, 41.4; H, 4.2; N, 19.3; S, 11.0.
Found: C, 40.8; H, 4.6; N, 19.6; S, 11.0.

The *free base* was obtained by solution of the dihydrochloride (Ib) in water and addition of excess ammonia. The pale yellow solid so obtained was recrystallized from ethanol; m.p. 212–214° (dec.).

*Anal.* Calc'd for $C_{10}H_{10}N_4S$: C, 55.0; H, 4.6.
Found: C, 55.0; H, 4.7.

*2,3-Dihydro-4-methyl-2-(4-pyridylmethylenehydrazono)thiazole dihydrochloride* (Ic). Isonicotinaldehyde thiosemicarbazone (20 g.) with chloroacetone gave 33 g. of a yellow-orange product by the procedure used for picolinaldehyde thiosemicarbazone. The product was recovered by chilling the reaction solution and was crystallized from methanol containing a small quantity of concentrated hydrochloric acid; yield, 33 g.; m.p. 255–257°.

*Anal.* Calc'd for $C_{10}H_{10}N_4S \cdot 2HCl \cdot \frac{1}{2}H_2O$: C, 40.0; H, 4.4; N, 18.7.
Found: C, 39.6; H, 4.6; N, 18.6.

*2-Imino-3-(3-pyridylmethyleneamino)-4-thiazoline dihydrochloride* (IIb). Nicotinaldehyde thiosemicarbazone hydrochloride (20 g.), 25 g. of chloroacetal, 500 ml. of methanol, and 10 ml. of concentrated hydrochloric acid were refluxed for 16 hours, filtered, and the filtrate was concentrated to one-third volume. Addition of ether gave a yellow precipitate which was recrystallized by solution in methanol and addition of ether; yield, 22 g.; m.p. 214–215°.

*Anal.* Calc'd for $C_9H_8N_4S \cdot 2HCl$: N, 20.4. Found: N, 20.6.

*2-Imino-4-phenyl-3-(3-pyridylmethyleneamino)-4-thiazoline dihydrochloride* (IIc). Nicotinaldehyde thiosemicarbazone hydrochloride (32.4 g.) was heated with 24 g. of chloro-

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[1] All melting points are corrected.

[2] The e.m.f. in an alcoholic system is a better convention than *p*H which refers to aqueous systems.

acetophenone in 500 ml. of methanol for 16 hours. On cooling, the yellow product was separated by filtration and recrystallized from methanol; yield, 26 g.; m.p. 229–230°.

*Anal.* Calc'd for $C_{15}H_{12}N_4S \cdot 2HCl$: N, 15.9; Cl, 19.8.

Found: N, 16.2; Cl, 19.8.

*2,3-Dihydro-4-phenyl-2-(4-pyridylmethylenehydrazono)thiazole hydrochloride* (Id). Isonicotinaldehyde thiosemicarbazone (7.2 g.) and 6.5 g. of chloroacetophenone were refluxed in 200 ml. of methanol. The reaction product crystallized out and on cooling was filtered off. The yellow substance was recrystallized from a large volume of methanol; yield, 10 g.; m.p. 258–260°.

*Anal.* Calc'd for $C_{15}H_{12}N_4S \cdot HCl \cdot \frac{1}{2}CH_3OH$: C, 56.0; H, 4.5; N, 16.9.

Found: C, 56.7; H, 4.2; N, 16.9.

*1-Methyl-3-cyanopyridinium chloride.* 3-Cyanopyridine (100 g.) was dissolved in 250 ml. of methanol and the solution was saturated with methyl chloride at 25°. The solution was held at 100° for 4 hours under nitrogen at 1000 p.s.i. pressure. The reaction mixture then was concentrated to one-fourth of its volume and on cooling the product crystallized out. It was recrystallized from hot ethanol as a colorless, water-soluble material; yield, 56 g.; m.p. < 250°.

*Anal.* Calc'd for $C_7H_7ClN_2$: C, 54.3; H, 4.5.

Found: C, 54.2; H, 4.3.

*Nicotinaldehyde thiosemicarbazone methochloride stannous chloride complex.* A mixture of 1-methyl-3-cyanopyridinium chloride (35 g.), 850 ml. of dry Diethyl Carbitol,[3] and 345 g. of anhydrous $SnCl_2$ was saturated with hydrogen chloride gas with stirring until complete solution of the reactants occurred. The solution then was mixed with 850 ml. of boiling water containing 31 g. of thiosemicarbazide and after refluxing for 2 hours to complete the reaction the solution was cooled. A yellow solid separated which was recrystallized from boiling water; yield, 80 g.; m.p. 204–205° (dec.).

*Anal.* Calc'd for $C_8H_{11}ClN_4S \cdot SnCl_2$: S, 7.6. Found: S, 7.7.

*Thiosemicarbazone of nicotinaldehyde methochloride.* The thiosemicarbazone of nicotinaldehyde methochloride stannous chloride complex (76 g.) was dissolved in 2 l. of boiling water and the tin was removed with hydrogen sulfide gas. The filtered solution was concentrated to 50 ml., methanol and benzene were added, and the remaining water was removed by azeotropic distillation. The yellow product obtained was crystallized from methanol; yield, 16 g.; m.p. 230–231°.

*Anal.* Calc'd for $C_8H_{11}ClN_4S$: S, 13.9. Found: S, 13.5.

*Nicotinaldehyde thiosemicarbazone anthraquinone-2-carboxylate.* Nicotinaldehyde thiosemicarbazone (9 g.) was dissolved in ethanol and was added to 13.5 g. of 2-carboxyanthraquinone in ethanol at 70°. The yellow solution was concentrated; on cooling it crystallized and the yellow salt was recrystallized from hot ethanol; yield, 20 g.; m.p. 226° (dec.).

*Anal.* Calc'd for $C_7H_8N_4S \cdot C_{15}H_8O_4$: S, 7.4. Found: S, 7.3.

This compound exhibited the expected anti-tubercular activity of a salt of nicotinaldehyde thiosemicarbazone.

*2-Amino-5-(3-pyridyl)-1,3,4-thiadiazole.* Nicotinaldehyde thiosemicarbazone hydrochloride (50 g.) was dissolved in 500 ml. of 80° water and a solution of 90 g. of $FeCl_3 \cdot 6H_2O$ in 100 ml. of water was added. The solution was held at 80° for 4 hours and an excess of concentrated ammonium hydroxide was added. The suspension was boiled, charcoal was added to coagulate the ferrous hydroxide, and it was filtered. The filter cake was extracted twice using 500 ml. of boiling water for each extraction. The combined hot water solutions were concentrated to a small volume and on cooling a creamy-colored solid separated which was recrystallized from boiling water; yield, 8 g.; m.p. 230–232° (dec.).

*Anal.* Calc'd for $C_7H_6N_4S$: S, 18.0. Found: S, 18.1.

*Nicotinaldehyde guanylhydrazone hydrochloride stannous chloride complex.* A mixture of 3-cyanopyridine (30 g.), 200 g. of anhydrous fused $SnCl_2$, and 600 ml. of dry Diethyl Carbitol[3] was saturated with dry hydrogen chloride gas. After complete solution and standing for

[3] Trade name for the diethyl ether of diethylene glycol.

18 hours, 800 ml. of boiling water containing 90 g. of aminoguanidine bicarbonate was added. On cooling, 103 g. of a crude white precipitate separated. A small sample was recrystallized from boiling water; m.p. 196–211° (dec.).

*Anal.* Calc'd for $C_7H_9N_5 \cdot HCl \cdot SnCl_2$: N, 18.0. Found: N, 18.3.

*Nicotinaldehyde guanylhydrazone dihydrochloride.* Crude nicotinaldehyde guanylhydrazone hydrochloride stannous chloride complex (100 g.) was dissolved in 1 l. of boiling water and hydrogen sulfide gas was used to precipitate the tin. The solution freed of SnS was concentrated to 75 ml. and 10 ml. of concentrated hydrogen chloride and 2 l. of ethanol were added. On concentration to 150 ml. and cooling, 46 g. of a white crystalline material separated which was recrystallized from aqueous ethanol; m.p. 258–259°.

*Anal.* Calc'd. for $C_7H_9N_5 \cdot 2HCl$: N, 29.6. Found: N, 28.9.

*Nicotinaldehyde semicarbazone hydrochloride stannous chloride complex.* A Stephen reaction, as described above for the guanylhydrazone, was run using 31.2 g. of 3-cyanopyridine in the presence of 35 g. of semicarbazide hydrochloride. A colorless complex formed (86 g.) which was recrystallized from hot water; m.p. 198–200° (dec.).

*Anal.* Calc'd for $C_7H_9N_4O \cdot HCl \cdot SnCl_2$: N, 14.3. Found: N, 14.1.

Treatment of the complex with hydrogen sulfide gas and purification gave nicotinaldehyde semicarbazone, m.p. 213°, for which m.p. 213–214° has been reported (9).

*The attempted preparation of nicotinaldehyde (nitroguanyl) hydrazone by the Stephen reduction.* 3-Cyanopyridine (60 g.) in Diethyl Carbitol[3] was reduced as in the previous examples and boiling water containing 62 g. of 1-amino-3-nitroguanidine [m.p. 188° (10)] was added. A violent reaction took place. On cooling, 156 g. of nicotinaldehyde guanylhydrazone dihydrochloride stannous chloride complex separated, described above in the preparation of nicotinaldehyde guanylhydrazone dihydrochloride.

The complex was decomposed with hydrogen sulfide gas and 75 g. of *nicotinaldehyde guanylhydrazone* dihydrochloride was obtained; m.p. 256–258° (dec.). The mixture m.p. with nicotinaldehyde guanylhydrazone dihydrochloride (m.p. 258–259°) prepared from aminoguanidine was unchanged.

*Anal.* Calc'd for *nicotinaldehyde guanylhydrazone dihydrochloride*: $C_7H_9N_5 \cdot 2HCl$: C, 35.6; H, 4.7; N, 29.6; Cl, 30.1.
Found: C, 35.9; H, 4.7; N, 29.8; Cl, 29.7.

SUMMARY

A series of substituted thiazoles was prepared from the pyridylaldehyde thiosemicarbazones. Variations in the method of preparation led to isomeric structures. These had different biological activities.

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