methanol. Papergrams of the resulting mixture showed a series of non-reducing oligosaccharides including sucrose, raffinose, stachyose and higher compounds. Sucrose and raffinose were obtained in crystalline form from the mixture using the charcoal separation method.[4] After brief acid hydrolysis, the mixture was separated on a papergram and examined for reducing sugars (alkaline copper method, using phosphomolybdic acid to locate areas in which reduction has taken place) and ketoses (phloroglucinol–hydrochloric acid). Reducing sugars included fructose, glucose, planteobiose, melibiose and less mobile oligosaccharides. Ketoses included fructose and planteobiose only. Similar results were obtained with Ash manna (*Fraxinus ornus*, obtained from S. B. Penick and Co.), except that the original manna also contains reducing sugars including planteobiose.

Chromatographic Data.—Paper chromatograms were prepared using the multiple ascent technique[14] with Eaton and Dikeman paper No. 613 and the butanol–pyridine–water mixture 3:2:1.5.[14] Reducing sugars were located[15] by the copper spray followed by phosphomolybdic acid. Ketose-containing sugars were located by spraying with a saturated solution of phloroglucinol in 0.4 *N* hydrochloric acid followed by heating in an oven at 100° to bring out the ketose color test. The $R_f/(1 - R_f)$ values found were as follows: planteose, 0.20; raffinose, 0.22; planteobiose, 0.45; sucrose, 0.69; galactose, 0.69; glucose, 0.89; fructose, 1.1.

(14) A. Jeanes, C. S. Wise and R. J. Dimler, *Anal. Chem.*, **23**, 415 (1951).

(15) D. French, D. W. Knapp and J. H. Pazur, THIS JOURNAL, **72**, 5150 (1950).

AMES, IOWA

[CONTRIBUTION FROM THE SCHENLEY LABORATORIES, INC.]

Antituberculous Compounds. III. Benzothiazole and Benzoxazole Derivatives

BY LEON KATZ

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Derivatives of 2-hydrazinobenzothiazole and 2-hydrazinobenzoxazole have been synthesized for testing as antituberculous agents. In addition, four compounds containing the benzothiazole nucleus and capable of complexing cupric ions have been synthesized. Although none of the compounds possessed outstanding antituberculous properties the derivatives of 2-hydrazinobenzoxazole had significant antifungal activity.

In a previous communication[1] three series of compounds prepared from 2-hydrazinobenzothiazoles were described. The present paper reports other derivatives of 2-hydrazinobenzothiazole as well as derivatives of the isosteric 2-hydrazinobenzoxazole.

TABLE I

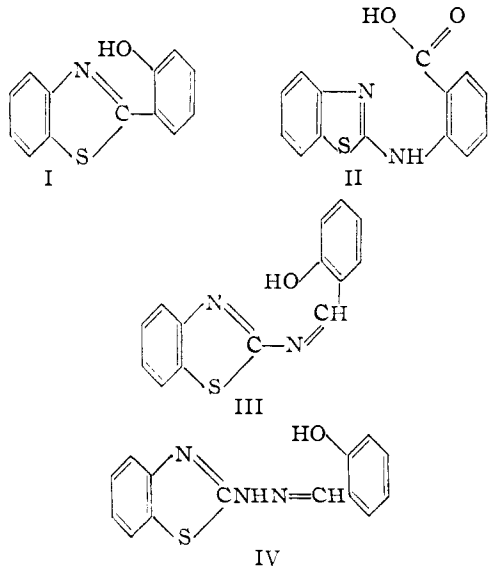
HYDRAZONES OF 2-HYDRAZINOBENZOTHIAZOLE

Carbonyl component	Yield, %	M.p., °C.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Benzalacetone	98	180–182[a]	$C_{17}H_{15}N_3S$	69.62	69.34	5.12	5.01	14.33	14.03
Anisalacetone	94	144–146[e]	$C_{18}H_{17}ON_3S$	66.87	66.90	5.26	5.00	13.00	12.67
Salicylideneacetone	99	177–179[b]	$C_{17}H_{15}ON_3S$	66.02	66.18	4.85	4.91	13.59	13.77
p-Dimethylaminobenzalacetone[f]	86	189–192[c]	$C_{19}H_{20}N_4S$	67.86	68.06	5.95	5.93	16.67	16.76
Salicylaldehyde	89	257[d]	$C_{14}H_{11}ON_3S$	62.45	62.74	4.08	4.15	15.61	15.60

Recrystallized from: [a] aqueous ethanol; [b] isopropyl alcohol; [c] isopropyl alcohol–ethylene chloride mixture; [d] glacial acetic acid; [e] aqueous acetic acid; [f] this compound was prepared by Dr. Horwitz following the procedure of H. Rupe, A. Collin and L. Schmiderer, *Helv. Chim. Acta*, **14**, 1340 (1931).

The recent disclosure that the thiosemicarbazone of benzalacetone[2] possessed outstanding antitubercular activity prompted us to condense 2-hydrazinobenzothiazole with α,β-unsaturated ketones. These hydrazones are included in Table I.

Several recent reports[3] have hypothesized that the antitubercular action of such drugs as 8-hydroxyquinoline, *p*-aminosalicyclic acid, Tibione[4] and the halogenated diphenyl ethers is due to their ability to complex cupric ions. Sorkin[5] and coworkers demonstrated that the antitubercular activity of 8-hydroxyquinoline was increased significantly (25–40 fold) in the presence of cupric ions. As a consequence, four compounds (I–IV), containing the benzothiazole moiety were synthesized



(1) L. Katz, THIS JOURNAL, **73**, 4007 (1951).

(2) H. Frahm and A. Lembke, *Zent. Bakt., I Abt.*, **154**, (8) 315 (1949).

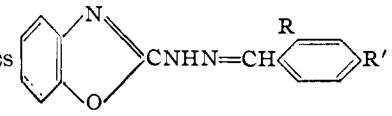
(3) E. Carl and P. Marquardt, *Z. Naturforschg.*, **46**, 280 (1949); K. Liebermeister, *ibid.*, **56**, 79, 254 (1950).

(4) "Tibione" is the Schenley registered trade-mark for *p*-acetylaminobenzaldehyde thiosemicarbazone known generically as Amithiozone.

(5) E. Sorkin, W. Roth and H. Erlenmeyer, *Experientia*, **7**, 64 (1951); E. Sorkin and W. Roth, *Helv. Chim. Acta*, **34**, 427 (1951). The latter reference contains an excellent group of references pertaining to the action of metal ions on the bacteriological properties of various compounds.

TABLE II

BENZALHYDRAZINOBENZOXAZOLES



R	R'	Yield, %	M.p., °C.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H	OH	81	249–250[a]	$C_{14}H_{11}O_2N_3$	66.40	66.12	4.35	4.06	16.60	16.62
H	OCH_3	89	207[b]	$C_{15}H_{13}O_2N_3$	67.42	67.62	4.87	4.96	15.73	15.56
H	OCH_2CO_2H	88	228–229[c]	$C_{16}H_{13}O_4N_3$	61.73	61.19	4.18	4.21	13.50	13.32
OH	H	85	250–251[a]	$C_{14}H_{11}O_2N_3$	66.40	66.08	4.35	4.26	16.60	16.90
OCH_3	H	93	233[d]	$C_{15}H_{13}O_2N_3$	67.41	67.68	4.87	4.69	15.73	15.66
OC_2H_5	H	86	185[b]	$C_{16}H_{15}O_2N_3$	68.33	68.41	5.34	5.50	14.95	15.01
H	$N(CH_3)_2$	83	223–224[d]	$C_{16}H_{16}ON_4$	68.57	68.29	5.71	5.68	20.00	20.00
H	$N(C_2H_5)_2$	84	199–200[d]	$C_{18}H_{20}ON_4$	70.13	70.03	6.49	6.51	18.18	18.30
H	$NHCOCH_3$[e]	90	294–295[c]	$C_{16}H_{14}O_2N_4$	65.31	65.18	4.76	4.70	19.05	18.97
H	Cl	91	248–249[d]	$C_{14}H_{10}ON_3Cl$	61.88	62.17	3.68	3.92	15.47	15.05
Cl	H	92	236[d]	$C_{14}H_{10}ON_3Cl$	61.88	62.14	3.68	3.76	15.47	15.29
H	$SO_2C_2H_5$	88	225.5–226[c]	$C_{16}H_{15}O_3N_3S$	58.36	58.42	4.56	4.61	12.76	13.01
H	CO_2H	61	281–282	$C_{15}H_{11}O_3N_3$	64.06	64.29	3.92	4.14		

Recrystallized from: [a] aqueous methyl cellosolve; [b] isopropyl alcohol; [c] aqueous dimethylformamide; [d] *n*-butanol; [e] The recrystallized solid was white and slowly changed to pink when exposed to light.

TABLE III

MISCELLANEOUS HYDRAZONES OF 2-HYDRAZINOBENZOXAZOLE

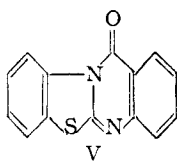
Carbonyl component	Yield, %	M.p., °C.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Cinnamaldehyde[f]	88	216[a]	$C_{16}H_{13}ON_3$	73.00	73.01	4.94	4.94	15.97	16.15
α-n-Amylcinnamaldehyde[f]	85	168–169[a]	$C_{21}H_{23}ON_3$	75.67	75.63	6.91	6.82	12.61	12.62
o-Hydroxyacetophenone	90	174–176[b]	$C_{15}H_{13}O_2N_3$	67.41	67.31	4.87	4.73	15.73	15.99
Benzalacetone[f]	80	180–184[c]	$C_{17}H_{15}ON_3$	73.65	73.50	5.42	5.14	15.16	14.95
Anisalacetone[f]	84	183–187[c]	$C_{18}H_{17}O_2N_3$	70.35	70.52	5.54	5.50	13.68	13.45
Salicylideneacetone[g]	83	160–162[d]	$C_{17}H_{15}O_2N_3$	69.62	69.46	5.12	5.21	14.33	14.03
p-Dimethylaminobenzalacetone[g]	89	190–191[e]	$C_{19}H_{20}ON_4$	71.25	71.46	6.25	6.29	17.50	17.61

Recrystallized from: [a] *n*-butanol; [b] aqueous ethanol; [c] aqueous methanol; [d] aqueous dimethylformamide; [e] isopropyl alcohol–ethylene chloride; [f] the solid slowly changed from white to pink in the light; [g] the original yellow color changed to orange-brown in the light.

since it was felt they would be capable of forming complexes with metallic ions.

The 2-(2′-hydroxyphenyl)-benzothiazole (I) was obtained from salicylaldehyde and 2-aminobenzenethiol; the anil of 2-aminobenzothiazole and salicylaldehyde (III), was prepared by refluxing one mole of the amino compound and two moles of the aldehyde in toluene; and 2-salicylalhydrazinobenzothiazole (IV) was obtained by the reaction of salicylaldehyde with 2-hydrazinobenzothiazole.

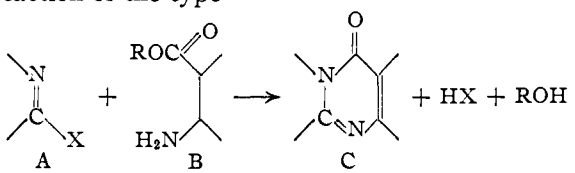
When 2-chlorobenzothiazole and anthranilic acid were heated together, a vigorous reaction occurred but the anticipated product, II, was not isolated. Instead an alkali-insoluble compound was obtained which analyzed correctly for the ring closed



V

product V, $C_{14}H_8ON_2S$. A search of the literature revealed that this compound was first obtained by Bose and Pathak[6] using the same reaction conditions.

When ethyl anthranilate was substituted for anthranilic acid and the reactants diluted with butanol in order to modify the vigorous reaction, V was the only isolated product. This ring closure has been extended to 2-chlorobenzoxazole and 2-chlorothiazole. Based on our experience and that of other investigators[7] this would appear to be a general reaction of the type[8]



A B C

Compound II was obtained by cleavage of V with dilute aqueous methanolic potassium hydroxide and acidification of the cold reaction mixture with dilute hydrochloric acid. In a qualitative manner it was demonstrated that compounds I–IV formed copper complexes.

2-Hydrazinobenzoxazole was obtained in 93% yield by treating 2-chlorobenzoxazole with hydrazine hydrate. The benzoxazole-2-hydrazones resulting from the condensation with aromatic car-

(6) P. K. Bose and K. B. Pathak, *J. Ind. Chem. Soc.*, **11**, 463 (1934).

(7) (a) P. K Bose and D. C. Sen, *J. Chem. Soc.*, 2840 (1931), condensed 2-chlorolepidine and 2-chloropyridine with anthranilic acid; (b) A. Reissert, *Ber.*, **28**, 119 (1898), heated 6-chloronicotinic acid and anthranilic acid together. The resulting product was decarboxylated and O. Seide, *Ann.*, **440**, 311 (1924), correctly characterized the decarboxylated material as a pyridoquinazolone.

(8) A related reaction between 2-aminopyridine and ethyl β-aminocrotonate leading to compounds of type C has been described. Other heterocyclic amino compounds can be employed. A leading reference is the recent paper by H. Antaki and V. Petrow, *J. Chem. Soc.*, 551 (1951).

bonyl compounds are listed in Tables II and III. These compounds, in particular those derived from α,β-unsaturated ketones, are light sensitive.

Although these compounds possessed definite *in vitro* antitubercular activity, no pronounced *in vivo* effectiveness could be demonstrated. It is noteworthy to point out that the benzoxazole derivatives were effective fungicidal agents, whereas the benzothiazole analogs showed little antifungal activity.

Acknowledgment.—The author is indebted to Dr. A. G. Karlson of the Mayo Foundation for the *in vivo* testing of two of the benzoxazole derivatives against *M. tuberculosis*.

Experimental[9]

Reaction of 2-Hydrazinobenzothiazole with Benzalacetone.—This reaction is cited as an example of the method employed for the preparation of the compounds listed in Table I. Into a 400-ml. beaker on a hot-plate were charged 70 ml. of isopropyl alcohol, 5 ml. of glacial acetic acid and 4.95 g. (0.03 mole) of 2-hydrazinobenzothiazole. The slurry was heated to boiling and a clear solution was obtained. To the boiling solution was added 4.4 g. (0.03 mole) of benzalacetone. The mixture was boiled for five minutes, while stirring manually, and allowed to cool to room temperature. In some cases the product crystallized from the boiling solution. The solid was collected on a buchner funnel, washed with 200 ml. of 50% aqueous methanol and dried at 60–65°. The weight of material, m.p. 176–178°, was 8.7 g. (98%).

These hydrazones did not crystallize well and the melting points were not sharp. This is undoubtedly due to the presence of two or more of the four possible *cis–trans* isomers.

2-Hydrazinobenzoxazole.—This compound was originally prepared by Bayer, Herdieckerhoff and Schindhelm[10] from benzoxazole-2-sodium sulfonate and hydrazine hydrate. The present procedure employs 2-chlorobenzoxazole. Into a 400-ml. beaker equipped with a stirrer, thermometer, dropping funnel and ice-bath was charged 60 g. (1.0 mole) of 85% hydrazine hydrate. Into the dropping funnel were placed 27.0 g. (0.175 mole) of 2-chlorobenzoxazole and 25 ml. of dioxane. This mixture was added to the hydrazine hydrate at such a rate that the temperature did not exceed 30°. Vigorous stirring was maintained throughout the addition. After the addition was complete, stirring was continued for 15 minutes and the slurry was diluted with 150 ml. of water. The solid was collected on a buchner funnel, sucked dry and washed with copious amounts of water to remove excess hydrazine. After drying at 65° overnight the weight of material, m.p. 150–152° (lit. 154–155°), was 24.5 g. (93%).

Reaction of 2-Hydrazinobenzoxazole with Carbonyl Compounds.—The methods described previously[1] and above were followed for the preparation of the compounds listed in Tables II and III.

2-(2′-Hydroxyphenyl)-benzothiazole (I).—An extension of a method used by Stephens[11] and Wibberley for the preparation of benzothiazoles from *o*-aminobenzenethiosulfuric acid and aldehydes was used. Into a 300-ml. flask equipped with Glas-Col heating mantle and reflux condenser were charged 12.5 g. (0.1 mole) of 2-aminobenzenethiol, 12.2 g. (0.1 mole) of salicylaldehyde, 100 ml. of glacial acetic acid and 20.0 g. (0.26 mole) of ammonium acetate. This mixture was refluxed 3.5 hours, poured into a beaker and cooled to 10°. The resulting precipitate was collected on a buchner funnel, washed with water and dried at 65°. The weight of white solid, m.p. 125–127.5°, was 13.3 g. (58%). No attempt was made to increase this yield. A sample recrystallized from ethanol melted at 131–132°.

Anal. Calcd. for $C_{13}H_9ONS$: C, 68.72; H, 3.96; N, 6.17. Found: C, 68.67; H, 3.82; N, 6.11.

Ten milligrams of I was dissolved in three drops of pyridine and 5 ml. of alcohol. When a drop of 1% copper sulfate solution was added a brown precipitate was formed. The blank remained blue.

Lankelma and Sharnoff[12] described the preparation of benzothiazolines by the condensation of 2-aminobenzenethiol with aldehydes in pyridine. The benzothiazolines are readily oxidized to benzothiazoles by ferric chloride or by recrystallization from ethanol. In a reaction using salicylaldehyde, 2-aminobenzenethiol and pyridine, an 81% yield of material, m.p. 113–120°, was obtained. Repeated recrystallization from ethanol yielded a compound, m.p. 130–131°, which did not depress the melting point of 2-(2′-hydroxyphenyl)-benzothiazole.

Reaction of 2-Aminobenzothiazole with Salicylaldehyde (III).—Into a 500-ml. flask equipped with a Glas-Col and water separator were charged 15.0 g. (0.10 mole) of 2-aminobenzothiazole, 12.5 g. (0.10 mole) of salicylaldehyde and 150 ml. of toluene. This mixture was refluxed vigorously for five hours. Inasmuch as only 0.5 ml. of water had been removed, an additional 12.5 g. (0.10 mole) of salicylaldehyde was added and the yellow solution refluxed an additional 15 hours at the end of which time the theoretical amount of water had been collected. The reaction mixture was chilled to 5° and the yellow precipitate collected and dried at 70°. From the filtrate a second crop was obtained by concentration to 50 ml. The total yield was 24.3 g. (95%), m.p. 139–142°. A sample was recrystallized twice from ethanol, m.p. 145.5–146°.

Anal. Calcd. for $C_{14}H_{10}ON_2S$: C, 66.14; H, 3.94; N, 11.02. Found: C, 66.06; H, 3.83; N, 10.99.

Ten milligrams of II was dissolved in 5 ml. of 50% aqueous acetic acid. When five drops of a saturated aqueous copper sulfate solution was added a blue-green solution resulted. The blank was a deep blue.

The same test was applied to IV and a yellow-green color developed.

N-(2-Benzothiazolyl)-anthranilic Acid (II).—Into a 100-ml. flask equipped with a Glas-Col and a reflux condenser were charged 5.1 g. (0.03 mole) of 2-chlorobenzothiazole, 4.95 g. (0.03 mole) of ethyl anthranilate and 50 ml. of glacial acetic acid. This mixture was heated under reflux for 45 minutes and the resulting yellow solution was poured into a beaker. Upon chilling to 10° a solid mass of needles was obtained. The solid was collected on a buchner funnel and washed with 100 ml. of methanol. After drying at 60°, 5.8 g. of V, m.p. 188–190°, was obtained. From the filtrate an additional 1.0 g. was recovered by dilution with an equal volume of water. The combined yield was 90%. A sample recrystallized several times from toluene–cyclohexane melted at 193° (lit.[6] 189°).

Into a 300-ml. flask equipped for reflux were charged 100 ml. of 50% aqueous methanol, 5.0 g. (0.02 mole) of V and 3.0 g. (0.052 mole) of potassium hydroxide. This mixture was refluxed gently for 2.5 hours. The clear yellow solution was transferred to a beaker, chilled to 0–5° and acidified to congo red with 6 *N* hydrochloric acid. The bulky white precipitate was collected on a filter and washed with water until the washings were acid-free to congo red. The weight of material, m.p. 194–196°, was 5.1 g. (98%). A sample was purified by dissolving in aqueous ammonia, reprecipitating with 3 *N* hydrochloric acid, drying and subsequently recrystallizing from a toluene–low-boiling petroleum ether mixture. Fine white needles, m.p. 195–196°, were obtained (lit.[6] 187°).

Anal. Calcd. for $C_{14}H_{10}O_2N_2S$: C, 62.22; H, 3.70; N, 10.37. Found: C, 62.23; H, 3.67; N, 10.14.

Ten milligrams of the acid was dissolved in 3 drops of pyridine and 5 ml. of ethanol. A green color was obtained upon addition of 1% copper sulfate solution, whereas the blank remained blue.

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(9) All melting points are uncorrected.

(10) O. Bayer, E. Herdieckerhoff and H. Schindhelm (to I. G. Farbenind. A-G) U. S. Patent 2,073,600 (Mar. 16, 1937).

(11) F. F. Stephens and D. G. Wibberley, *J. Chem. Soc.*, 3336 (1950).

(12) H. P. Lankelma and P. X. Sharnoff, THIS JOURNAL, **53**, 2654 (1931).