since the copper salts were either liquids or were very soluble in ligroin. Generally the β-diketones were isolated by fractionation as described above in Method B.

(B) **Lithium Amide.**—Commercial lithium amide[13] (0.6 mole) was suspended in 300 ml. of dry ether, and 0.6 mole of ketone in 50 ml. of dry ether was added. After refluxing for 15 minutes, a solution of 0.3 mole of the ester in 50 ml. of ether was added. Refluxing was continued for 3 hours and the reaction mixture was worked up as described above for acylations with sodium amide.

(C) Sodium Hydride.—Acylations with this reagent were carried out by the procedure described previously.[3]

(13) We are indebted to the Metalloy Corporation, Minneapolis, Minnesota, for a generous supply of lithium amide.

Copper Enolate Derivatives.—To a sample of the β-diketone obtained by fractionation (about 5 g.) dissolved in an equal volume of methanol was added 100 ml. of a saturated solution of copper acetate (40 g. of copper acetate hydrate in 350 ml. water), and the mixture allowed to cool. If the copper enolate solidified, it was filtered by suction and recrystallized from 95% ethanol. If the enolate did not solidify, it was extracted from the aqueous portion with ligroin (b.p. 30–60°), the ligroin evaporated and the residue recrystallized from 95% ethanol. A second recrystallization from ethanol yielded pure samples, the melting points of which are given in the notes of Table I. In several instances the enolates were liquid and attempts to obtain solid derivatives failed.

DURHAM, NORTH CAROLINA RECEIVED AUGUST 11, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, PARKE, DAVIS & CO.]

Some Derivatives of 4-Amino-2-hydroxybenzoic Acid (*p*-Aminosalicylic Acid)

BY LEONARD DOUB, J. J. SCHAEFER, L. L. BAMBAS AND CONSTANCE T. WALKER

A number of derivatives and analogs of 4-amino-2-hydroxybenzoic acid have been prepared for tuberculostatic test. None of those tested was as active as the parent compound either *in vitro* or *in vivo*.

The versatile intermediates 2-acetoxy-4-nitrobenzoyl chloride and 2-hydroxy-4-nitrobenzimino ether hydrochloride have been prepared and characterized.

Following the announcement by Lehman[1] of the effectiveness of *p*-aminosalicylic acid (PAS) in tuberculosis we prepared several derivatives of this compound to explore the possibility of improving its activity.[2]

The N-alkylated compounds (Table I, nos. 8, 9, 10, 11) were prepared by application of a modified Kolbe procedure on the appropriately substituted *m*-aminophenol. The orientation is assumed by analogy with the formation of PAS by the same process. Structure is confirmed in the case of the N-methyl derivative in that the compound from this procedure is identical with that obtained by methylation of PAS.[3]

The amidines (nos. 18, 19) were made by the catalytic reduction of the corresponding nitro compounds. These latter were in turn prepared from 2-hydroxy-4-nitrobenzonitrile through the imino ether (no. 36).

We were able to prepare in good yield the intermediate 2-acetoxy-4-nitrobenzoyl chloride. Reaction of this with the appropriate amines followed by reduction led to the amides listed in Table I (nos. 12, 13, 14, 15). A number of these are available by reaction of the amines with esters of PAS or 2-hydroxy-4-nitrobenzoic acid.[4,5] The chloride has the advantage of course that it readily reacts with weak amines and also can be used in Schotten–Baumann procedures. In this respect an attempt was made to prepare in this series the analogs of sulfathiazole and sulfadiazine. Condensation of the acid chloride with the aminoheterocycles was successful (nos. 34, 35) but due to the extraordinary insolubility of the amino compounds the reduction and purification were not completed. It was not determined whether the nitrohydroxybenzoyl moiety was attached to the amino group or the ring nitrogen of the heterocycles.

In an attempt to obtain amides directly from PAS which is more available than the nitro acid, we prepared 4-carbethoxyamino-2-hydroxybenzoyl chloride. This intermediate reacted readily with amines and alcohols (nos. 27, 28, 29) but attempts to hydrolyze preferentially the carbethoxy group were unsuccessful.

The bacteriostatic activities[6] of the derivatives listed in Table I in no case equal and in only a few cases approach that of the parent PAS. The appreciable activity of no. 8 may be a reflection of the ready metabolism of N-methyl groups generally,[7,8] whereby PAS is generated. With this exception, substitution of the amino group results in drastic loss of *in vitro* activity. Similarly it appears that a free hydroxyl group is necessary. Variation of the carboxyl group with the exception of esterification results in greatly reduced activity. The high activity of the glycine amide (no. 14) is only apparent since it is abolished in the presence of serum. It would appear possible that the high activity of the methyl ester (no. 16) might arise because of hydrolysis to PAS in the course of the fourteen-day duration of the *in vitro* test.

Compounds nos. 2, 5, 6, 8, 12, 16, 17, 21, 22 and 23 were tested in mouse tuberculosis.[6] These were essentially inactive except with nos. 5 and 17 where some slight activity was evident on the basis of full activity for PAS.

These data taken in conjunction with other re-

(1) Lehman, *Lancet*, **250**, 15 (1946).

(2) While this work was in progress some of these derivatives, especially esters and amides, have been reported by other workers. Representatives of these classes of compounds have been included in the present report, however, in order to present a more complete picture of the effect of structure on activity.

(3) Rosdahl, *Svensk Kem. Tid.*, **60**, 12 (1948).

(4) Jensen, Rosdahl and Ingvorsen, *Acta Chem. Scand.*, **2**, 220 (1948).

(5) Schaefer and Doub, THIS JOURNAL, **71**, 3564 (1949).

(6) The data reported here, both *in vitro* and *in vivo*, were obtained by Dr. Guy P. Youmans, Department of Bacteriology, Northwestern University Medical School. The authors are deeply indebted to him for permission to use his results.

(7) Gordon and Jackson, *J. Biol. Chem.*, **110**, 153 (1935).

(8) Abbott and Lewis, *ibid.*, **131**, 479 (1939).

TABLE I

p-AMINOSALICYLIC ACID DERIVATIVES 

No.	R_1	R_2	R	M.p.,[a] °C.	Crystn. solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Tuberculostatic activity[b] in synthetic media Mg. %	Tuberculostatic activity[b] in synthetic media 10% serum added
1	NH_2	OH	CO_2H[c]	145–146 (dec.)	....	$C_7H_7NO_3$	..	..	...	...	9.15	9.14	0.078	(0.156)
2	OH	OH	CO_2H[d]	217–218 (dec.)	....	.....	..	..	...	...	..	..	10.0	−
3	NO_2	OH	CO_2H[e]	235 (dec.)	....	.....	..	..	...	...	..	..	5.0	+
4	CH_3CONH	OH	CO_2H[e]	233 (dec.)	Dioxane	$C_9H_9NO_4$	55 4	55 4	4.7	4.8	7.2	7.3	10.0	..
5	$C_2H_5CO_2NH$	OH	CO_2H	212 (dec.)	50% EtOH	$C_{10}H_{11}NO_5$	53.3	53.6	4.9	5.1	6.2	6.3	10.0	+
6	CH_3CONH	CH_3CO_2	CO_2H[e]	188–189 (dec.)	AcOH	$C_{11}H_{11}NO_6$	55.7	55.3	4.7	4.8	5.9	5.9	2.5	+
7	$C_2H_5CO_2NH$	CH_3CO_2	CO_2H	166 (dec.)	$MeNO_2$	$C_{12}H_{13}NO_6$	53.9	54.2	4.9	5.0	5.2	5.4	....	..
8	CH_3NH	OH	CO_2H[f]	125–126 (dec.)	[m]	$C_8H_9NO_3$	57.5	57.9	5.4	5.6	..	..	0.625	+
9	CH_2=$CHCH_2NH$	OH	CO_2H	132–134 (dec.)	[m]	$C_{10}H_{11}NO_3$	62.2	62.4	5.7	5.7	..	..	10.0	+
10	$C_6H_5CH_2NH$	OH	CO_2H	160–161 (dec.)	Dil. MeOH	$C_{14}H_{13}NO_3$	69.1	69.1	5.4	5.5	..	..	10.0	+
11	$(C_2H_5)_2N$	OH	CO_2H[g]	142–145 (dec.)	[m]	$C_{11}H_{15}NO_3$	63.1	62.9	7 2	7 1	..	..	10 0	..
12	NH_2	OH	$CONH_2$[e,h]	157–159	Water	.....	..	..	...	...	..	..	10.0	+
13	NH_2	OH	$CONHC_2H_5$[h]	142–143	Water	$C_9H_{12}N_2O_2$	60.0	59.9	6.7	6.4	15.6	16.0	....	..
14	NH_2·HCl	OH	$CONHCH_2CO_2H$	215 (dec.)	Ppt. from Et_2O	$C_9H_{10}N_2O_4HCl$	43.8	43.9	4.5	4.5	11.4	11.1	0.312	−
15	NH_2	OH	$CONHC_6H_5$	144–145	50% EtOH	$C_{13}H_{12}N_2O_2$	68.4	67.7	5.3	5.3	12.3	12.4	2.5	+
16	NH_2	OH	CO_2CH_3[f]	121–122	Water	$C_8H_9NO_3$	..	..	...	...	8.4	8.3	0.625	+
17	NH_2	OH	$CO_2C_2H_5$[f,h]	114	Abs. EtOH	$C_9H_{11}NO_3$	59.7	60.0	6.1	6.4	7.7	7.5	2.5	−
18	NH_2·HCl	OH	$C(NH)NH_2$·HCl	277–278 (dec.)	90% concd. HCl	$C_7H_9N_3O$·2HCl	37.5	37.6	5.0	5.0	18.8	18.7	10.0	−
19	NH_2·HCl	OH	$C(NH)NHC_2H_5$·HCl	272 (dec.)	90% concd. HCl	$C_9H_{13}N_3O$·2HCl	42.9	42.9	6.0	6.0	16.7	17.0	10.0	−
20	NH_2	OH	SO_3H[i]	275 (dec.)	....	.....	..	..	...	...	..	..	10.0	..
21	NH_2	OH	SO_2NH_2[j]	151–153	....	.....	..	..	...	...	..	..	10.0	+
22	NH_2	SH	CO_2H[k]	211–213 (dec.)	....	.....	..	..	...	...	..	..	10.0	−
23	NH_2	CH_3O	CO_2H[l]	157–158	....	$C_8H_9NO_3$	57.5	57.1	5.4	5 6	..	..	10 0	−
24	NH_2	$H_2NC_6H_4S$	CO_2H	233–235 (dec.)	[m]	$C_{13}H_{12}N_2O_2S$	60.0	59.8	4.7	4.7	..	..	10.0	+
25	NO_2	CH_3CO_2	COCl	56–57	AcCl, pet. ether	$C_9H_6ClNO_5$	44.4	44.5	2.5	2.6	5.8	5.9	....	..
26	$C_2H_5CO_2NH$	OH	COCl	115 (dec.)	Et_2O	$C_{10}H_{10}ClNO_4$	49.3	50.0	4.1	4.0	5.8	5.9	....	..
27	$C_2H_5CO_2NH$	OH	CO_2CH_3	139–140.5	EtOH	$C_{11}H_{13}NO_5$	55.2	55.0	5.5	5.4	5.9	5.8	10.0	−
28	$C_2H_5CO_2NH$	OH	$CO_2C_2H_5$	146–148	EtOH	$C_{12}H_{15}NO_5$	56.9	56.8	6.0	6.0	5.5	5.6	....	..
29	$C_2H_5CO_2NH$	OH	$CONHC_6H_5$	187–188	Dil. EtOH	$C_{16}H_{16}N_2O_4$	64.0	64.3	5.4	5.5	9.3	9.5	5.0	..
30	NO_2	OH	$CONHC_2H_5$	157–158	50% EtOH	$C_9H_{10}N_2O_4$	51.4	51.1	4.8	4.8	..	..	....	..
31	NO_2	OH	$CONHCH_2CO_2H$	211–213 (dec.)	Water	$C_9H_8N_2O_6$	45.0	44.9	3.4	3.6	11.7	11.5	....	..
32	NO_2	OH	$CONHCH(CO_2H)CH_2CH_2CO_2H$	171–172 (dec.)	Et_2O PhH	$C_{12}H_{12}N_2O_8$	46.2	46.3	3.9	3.8	..	..	....	..
33	NO_2	OH	$CONHC_6H_5$	234–235 (dec.)	50% EtOH	$C_{13}H_{10}N_2O_4$	60.5	60.4	3.9	3.9	..	..	2.5	..
34	NO_2	OH	$CO(C_4H_4N_2)$	289 (dec.)	$PhNO_2$	$C_{11}H_8N_4O_4$	50.8	50.7	3.1	3.2	21.5	21.2	....	..
35	NO_2	OH	$CO(C_3H_3N_2S)$	306 (dec.)	$PhNO_2$	$C_{10}H_7N_3O_4S$	45.3	45.5	2.7	2.7	15.1	15.4	2.5	+
36	NO_2	OH	$C(NH)OC_2H_5$·HCl	190 (dec.)	....	$C_9H_{10}N_2O_4$·HCl	43.8	43.7	4.5	4.6	11.4	11.5	....	..
37	NO_2	OH	$C(NH)NH_2$	300 (dec).	See prep.	$C_7H_7N_3O_3$	46 4	46.6	3.9	3.8	23.2	23.3	10.0	−
38	NO_2	OH	$C(NH)NHC_2H_5$	218–220 (dec.)	50% EtOH	$C_9H_{11}N_3O_3$	51.7	51.9	5.3	5.5	20.1	20.6	10.0	−
39	NO_2	*p*-O_2N-C_6H_4-S	CO_2H	214–216	MeOH	$C_{13}H_8N_2O_6S$	48.8	49.0	2.5	2.6	8.8	8.8	....	..
40	NO_2	CH_3CO_2	CO_2H	156–156.5	PhH	$C_9H_7NO_6$	48.0	48.3	3.1	3.3	..	..	....	..

[a] Uncorrected. The decomposition points listed are extremely dependent on the rate of heating. These were obtained by immersing the capillary tube in the preheated-bath at approximately 10° below the melting point and bringing up to temperature rapidly. [b] This information kindly supplied by Dr. Guy P. Youmans, Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois. The concentration given is that determined by serial dilution in media which just prevents growth of the tubercle bacillus strain H37Rv. + Denotes active; − denotes inactive, depending upon whether complete inhibition of growth occurs at 10 mg. % concentration. [c] Seidel and Bittner, *Monatsh.*, **23**, 415 (1902); Kondo, *et al.*, *J. Pharmacol. Soc. (Japan)*, **483**, 355 (1922). [d] "Organic Syntheses," Coll. Vol. II, second edition, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 557. [e] Reference 11. [f] Reference 3. [g] Heyden, German Patent 50,835 (1890). [h] Reference 4. [i] Thorpe and Williams, *Biochem. J.*, **35**, 61 (1941). [j] Supplied by Dr. H. S. Mosher, Department of Chemistry, Stanford University, Palo Alto, California. [k] Feldt and Fritzsche, U. S. Patent 1,207,284 (1917). [l] Froelicher and Cohen, *J. Chem. Soc.*, 121, 1652 (1922). [m] These compounds, due to instability or other reasons, were prepared for analysis by repeated solution in bicarbonate and precipitation by acid, followed by careful washing in water.

ports which have appeared[9] point up the decided structural specificity associated with PAS activity. Considering further that this action apparently is shown only against tubercle bacilli[10] it constitutes a remarkable example of specificity in chemotherapy.

Experimental

All melting points uncorrected.

4-Acetamino-2-hydroxybenzoic Acid.—4-Acetamino-2-acetoxybenzoic acid[11] (9.4 g.) was dissolved in 100 ml. of *N* NaOH. After standing ten minutes the solution was filtered, cooled, and acidified strongly with dilute hydrochloric acid. The solid was filtered off and dried over phosphorus pentoxide at *ca.* 1 mm. pressure for sixty hours; yield, 7.6 g., 98%; m.p. 231–232° dec.

2-Acetoxy-4-nitrobenzoic Acid.—2-Hydroxy-4-nitrobenzoic acid (80 g.) was suspended in 100 ml. of acetic anhydride. This was slowly brought to boil and refluxed until the solution was clear. After the addition of charcoal the solution was filtered through a sintered glass filter. The filtrate on cooling deposited light yellow crystals; filtered, yield 53 g., m.p. 152–154°. The filtrate was concentrated to half its volume and then diluted with water until cloudy, cooled, filtered; yield 12 g., m.p. 151–154°; combined yield 65 g., 66%.

2-Acetoxy-4-nitrobenzoyl Chloride.—Phosphorus pentachloride (25 g.) was suspended in 103 ml. of acetyl chloride. 2-Acetoxy-4-nitrobenzoic acid (20 g.) was added in *ca.* 5-g. portions. Reaction ensued immediately after each addition. After all of the acid was added, the mixture was refluxed 1 hour. After cooling to room temperature, 100 ml. of dry petroleum ether was added. The solution was cooled in a Dry Ice–alcohol-bath; crystals formed on scratching. Filtered and washed with petroleum ether; yield 1st crop, 14 g., m.p. 57°. To the filtrate 50 ml. of petroleum ether was added and again cooled in a Dry Ice-bath, filtered; yield 5 g., m.p. 55–57°; combined yield 19 g., 88%.

4-Nitro-2-(4′-nitrophenylmercapto)-benzoic Acid.—2-Amino-4-nitrobenzoic acid[12] (37 g.) was diazotized by the invert method with 15 g. of sodium nitrite in 100 ml. of concd. hydrochloric acid. This was added to a warm solution prepared from 60 g. of sodium *p*-nitrothiophenol and 100 g. of sodium hydroxide in 500 ml. of water. The resulting solution was heated to boiling to complete reaction, cooled and the precipitate removed by filtration. The product was purified by solution in water, filtering from insoluble material, and reprecipitation with excess concd. hydrochloric acid. This product was dissolved in dilute bicarbonate solution and the preceding purification repeated. The resulting precipitate was crystallized from dilute methanol. The yield of light yellow crystalline solid was 6.8 g.

4-Carbethoxyamino-2-hydroxybenzoic Acid.—PAS (75 g.) was suspended in 300 ml. of water, 40 g. of sodium hydroxide in 200 ml. of water was added, and the solution cooled to 5°. Ethyl chlorocarbonate (125 g.) was added slowly with intermittent addition of 10 *N* NaOH as needed to keep slightly alkaline to phenolphthalein. The temperature was kept below 10° by external cooling and by addition of ice if necessary. This was diluted to 1 liter and warmed to approximately 20° to obtain a clear solution. On acidifying strongly the product precipitated, was filtered off and washed with water; yield 116 g. (quantitative), m.p. 202–204° dec.

4-Carbethoxyamino-2-hydroxybenzoyl Chloride.—4-Carbethoxyamino-2-hydroxybenzoic acid (225 g.) was suspended in 100 ml. of thionyl chloride and slowly heated on a steam-bath until clear, then refluxed until no more gas was evolved. The solution, after the addition of charcoal, was filtered, then cooled to crystallization and filtered; yield 175 g., 72%; m.p. 108–110° (dec.). A second crop was obtained by evaporation to dryness, dissolving in ether, treatment with charcoal, filtering and again evaporating to dryness; yield 60 g., 25%; m.p. 106–110° (dec.).

2-Acetoxy-4-carbethoxyaminobenzoic Acid.—2-Hydroxy-4-carbethoxyaminobenzoic acid (50 g.) was heated on the steam-bath with 250 ml. of acetic anhydride containing 1 ml. of pyridine until a clear solution was formed; cooled to crystallization, filtered; yield 58 g. (quantitative); m.p. 164–165° (dec.).

2-Hydroxy-4-nitrobenzimino Ethyl Ether Hydrochloride.—2-Hydroxy-4-nitrobenzonitrile[13] (3.3 g.) was dissolved in 10 ml. of absolute ether and 3 ml. of absolute ethyl alcohol containing 1.8 g. of anhydrous hydrogen chloride. This was allowed to stand in a refrigerator (*ca.* 4°) for 5 days, filtered, and washed with anhydrous ether; yield 4.5 g., 91%; m.p. 190° (dec.).

2-Hydroxy-4-nitrobenzamidine Hydrochloride.—2-Hydroxy-4-nitrobenzimino ethyl ether hydrochloride (12.5 g.) was added to 25 ml. absolute ethanol containing 3 g. of anhydrous ammonia. The temperature was kept below 40° and the solution was allowed to stand two days, evaporated to dryness under vacuum, and crystallized from 50% hydrochloric acid; yield 10 g., 91%, m.p. above 300°. For analysis a sample of the hydrochloride was dissolved in water and precipitated with ammonia hydroxide. The free base was washed several times with water and dried. The product melted above 300° and was a deep red compound, very insoluble in all common solvents

N-Ethyl-2-hydroxy-4-nitrobenzamidine.—Imino ether hydrochloride (12.5 g.) was added to 25 ml. of absolute ethyl alcohol containing 25 ml. of ethylamine and allowed to stand two days. This was evaporated to dryness under vacuum, dissolved in water, treated with charcoal and filtered. The solution, on being made alkaline to *p*H 9 with ammonium hydroxide and cooling, deposited crystals. These were filtered off and recrystallized from dilute alcohol; yield 9 g., 85%, m.p. 222–223° (dec.).

General Procedures. I. Kolbe–Schmidt Synthesis. (a) Aqueous Modification.—The following procedure for the preparation of PAS is convenient in that the use of pressure is avoided. The yield is moderate compared to that obtainable by application of carbon dioxide pressure.

m-Aminophenol (436.5 g.) and 2 kg. of potassium bicarbonate, after mixing, were covered with 5.2 l. of water in a closed flask fitted with a condenser, an inlet tube, and an exit tube. Under a slight positive exit pressure (30 cm. water) carbon dioxide was bubbled through the solution. The mixture was heated on the steam-bath (internal temperature 96°) for 90 hours; cooled and acidified with a large excess of concd. HCl; yield of white-to-gray hydrochloride, 38–41%; m.p. 221–223° (dec.) (rapid heating).

The use of 30–50 p.s.i. carbon dioxide pressure led to 50–60% yields of comparable material.

Application of this procedure to the appropriately substituted *m*-aminophenol led to low yields (5–20%) of compounds nos. 8, 10, 11 (isolated as free compound, not as hydrochloride). The intermediate *m*-allylaminophenol for no. 9, prepared by the action of allyl bromide on *m*-aminophenol, could not be obtained in a state of satisfactory purity. The crude oil was submitted to this Kolbe procedure giving an acid which after extensive purification amounted to *ca.* 5% yield over-all.

In every case above the majority of unreacted *m*-aminophenol could be recovered by extraction.

I (b)—Compound 10 was obtained by the usual Kolbe–Schmidt process using the dry sodium salt of *m*-benzylaminophenol at 130° under 1500 p.s.i. carbon dioxide pressure for twelve hours; yield *ca.* 10%.

II. **Formation of Amides.**—The very reactive acid chlorides (2-acetoxy-4-nitrobenzoyl chloride and 4-carbethoxyamino-2-hydroxybenzoyl chloride) were readily converted to amides or esters by following usual procedures depending upon the amine or alcohol used: (a) By reaction with large excess of amine or alcohol. Compounds nos. 27, 28, 29, 30 and 33; yields essentially quantitative except no. 29 gave 63%. (b) By the Schotten–Baumann procedure with the acid chloride dissolved in ether or chloroform: compounds nos. 31 and 32; 98 and 74% yield, respectively. (c) By reaction with equivalent amounts of amine (aminoheterocycles) in pyridine: compounds nos. 34 and 35; yields 42 and 87%, respectively. (d) With compound no. 33 the acetoxy group was not removed when the reaction mixture was diluted with water prior to acidification. A step was introduced involving solution in excess dilute sodium hydroxide at room temperature. The compound dissolved immediately with loss of this group, and acidification recovered the hydroxyl compound.

(9) Lehman, *Svenska Läkartidn.*, **43**, 2029 (1946).
(10) Sievers, *ibid.*, **43**, 2041 (1946).
(11) Drain, *et al.*, *J. Chem. Soc.*, 1498 (1949).
(12) Blanksma and Hoegen, *Rec. trav. chim.*, **65**, 333 (1946).
(13) Borsche, *Ann.*, **390**, 1 (1912).

III. Reductions.—Compounds nos. 30, 31, 33 and 38 were reduced catalytically (Adams catalyst) in absolute alcohol at room temperature and *ca.* 4 atmospheres pressure. With compound no. 37 methanol–aqueous hydrochloric acid mixture was used as solvent. The yields in every case ran 76–87%.

Compound 39 was reduced with iron in dilute ammonium chloride. The filtered sludge was extracted with dilute sodium carbonate, and the product precipitated by acidification with hydrochloric acid; yield 51%.

DETROIT 32, MICHIGAN RECEIVED JULY 21, 1950

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

The Chemotherapy of Experimental Tuberculosis. III. The Synthesis of Thiosemicarbazones and Related Compounds[1,2]

BY JACK BERNSTEIN, HARRY L. YALE, KATHRYN LOSEE, MARY HOLSING, JOSEPH MARTINS AND W. A. LOTT

The preparation of a considerable number of thiosemicarbazones and related compounds which were to be tested for antituberculous activity is described. The majority of the compounds prepared were variously substituted derivatives of benzaldehyde thiosemicarbazone. The nuclear substituents included $CH_3CONH\cdot$, $HO_2CCH_2CH_2CONH\cdot$, $(CH_3)_2N\cdot$, $H_2N\cdot$, $O_2N\cdot$, $NC\cdot$, $NaO_3S\cdot$, $C_2H_5O_2S\cdot$, $H_2NO_2S\cdot$, $(C_2H_5)_2NCH_2CH_2O\cdot$; $HO\cdot$, $-OCH_2O-$, $HO_2C\cdot$, $CH_3\cdot$, $C_2H_5O\cdot$, $HO_2CCH_2O\cdot$, $CH_3O\cdot$, n-$C_3H_7O\cdot$, i-$C_3H_7O\cdot$, n-$C_4H_9O\cdot$, i-$C_3H_7\cdot$, t-$C_4H_9\cdot$, Cl and I groups; the lateral substituents included $CH_3\cdot$, $C_2H_5\cdot$, $CH_2{:}CHCH_2\cdot$ n-$C_4H_9\cdot$, i-$C_4H_9\cdot$, and $C_6H_5\cdot$ groups. Thiosemicarbazones were prepared also of a number of substituted cinnamaldehydes and acetophenones. To complete this phase of the chemical study, a number of aliphatic, alicyclic, heterocyclic and α,β-unsaturated aldehydes and ketones were converted to thiosemicarbazones. To ascertain both the extent and limitations of antituberculous activity, a number of related compounds were prepared.

The antituberculous activity, *in vitro*, of certain thiosemicarbazones of aromatic aldehydes and ketones was reported first by Domagk, Behnisch, Mietzsch and Schmidt.[3] In a subsequent paper,[4] these authors indicated qualitative differences in activity among various thiosemicarbazones. Prior to these publications, there had been initiated, in these laboratories, a thorough investigation into the chemotherapy of experimental tuberculosis. As a consequence, when these reports became available to us, we undertook the preparation of a number of thiosemicarbazones and related compounds in an attempt to show quantitatively the relationship between chemical structure and antituberculous activity. While our investigation was in progress, Hoggarth, Martin, Storey and Young[5] published their excellent quantitative *in vivo* evaluation of a considerable number of thiosemicarbazones and related compounds. The *in vitro* and *in vivo* antituberculous activities of some of the compounds prepared in these laboratories have been published recently[2]; this paper is concerned only with their synthesis and characterization.[6]

The majority of the compounds prepared were mono- and poly-substituted derivatives of benzaldehyde 3-thiosemicarbazone. The nuclear substituents included the $CH_3CONH\cdot$, $HO_2CCH_2CH_2CONH\cdot$, $(CH_3)_2N\cdot$, $H_2N\cdot$, $CH_2{:}CHCH_2NHCSNH\cdot$, $NC\cdot$, $NaO_3S\cdot$, $CH_3SO_2\cdot$, $C_2H_5SO_2\cdot$, n-$C_3H_7SO_2\cdot$, $(C_2H_5)_2NCH_2CH_2O\cdot$, $HO_2CCH_2O\cdot$, $CH_3O\cdot$, $C_2H_5O\cdot$, n-$C_3H_7O\cdot$, n-$C_4H_9O\cdot$, $HO\cdot$, $\cdot OCH_2O\cdot$, $HO_2C\cdot$, $CH_3\cdot$, i-$C_3H_7\cdot$, t-$C_4H_9\cdot$, F_3C, Cl and I groups; the lateral substituents included the $CH_3\cdot$, $C_2H_5\cdot$, $CH_2{:}CHCH_2\cdot$, n-$C_4H_9\cdot$, i-$C_4H_9\cdot$ and $C_6H_5\cdot$ groups. Thiosemicarbazones were prepared also of various aliphatic, alicyclic, α,β-unsaturated and heterocyclic carbonyl compounds as well as a number of acetophenones. A number of aldehydes otherwise unavailable were synthesized either by methods previously described in the literature or by methods described in the Experimental part. The carbonyl compounds were condensed with thiosemicarbazide in aqueous ethanol, often in the presence of a small amount of acetic acid. These compounds are listed in Tables I and II.

The amino-substituted benzaldehyde 3-thiosemicarbazones were prepared by the iron and acetic acid reduction of the corresponding nitro compounds. The reaction of 4-aminobenzaldehyde 3-thiosemicarbazone with allyl isothiocyanate gave the 4-allylthiourea derivative and the reaction with succinic anhydride gave the 4-succinoyl derivative.

The substituted thiosemicarbazides were prepared according to the procedure described by Pulvermacher[7] and condensed in similar fashion with the desired aldehydes. These derivatives are to be found in Table III.

A number of miscellaneous compounds structurally related to the thiosemicarbazones were also prepared and these are shown in Table IV. The ferric chloride oxidation[8] of 4-methoxy- and 4-aminobenzaldehyde 3-thiosemicarbazones gave the 5-substituted-2-amino-1,3,4-thiadiazole derivatives; the sodium amalgam reduction of the same thiosemicarbazones gave the correspondingly substituted 1-benzyl-3-thiosemicarbazides. 1-(4-Aminobenzoyl) thiosemicarbazide was prepared by the reduction of the corresponding nitro derivative[9]

(1) Presented before the Division of Medicinal Chemistry, 117th Meeting, American Chemical Society, Philadelphia, Pa., April 9–13, 1950.

(2) The previous papers in this series are: I. Donovick, Pansy, Stryker and Bernstein, *J. Bact.*, **59**, 667 (1950); II. Hamre, Bernstein and Donovick, *ibid.*, **59**, 675 (1950).

(3) Domagk, Behnisch, Mietzsch and Schmidt, *Naturwissenschaften*, **33**, 315 (1946).

(4) Behnisch, Mietzsch and Schmidt. *Angew. Chem.*, **60**, 113 (1948).

(5) Hoggarth, Martin, Storey and Young. *Brit. J. Pharmacol.*, **4**, 248 (1949).

(6) Some of the compounds described in this paper have been mentioned in German Patent Applications J 76,179; 76,180; 76,218; 76,219; 76,679; 76,680; 76,745; 77,783; 77,784; 78,133; 78,134; 78,163; and 78,658. Photostats of these applications are available from the Department of Commerce, Office of Publication, or from the Research Information Service, 509 Fifth Ave., New York 17, N. Y.

(7) Pulvermacher, *Ber.*, **27**, 622 (1894).

(8) Young and Eyre, *J. Chem. Soc.*, **79**, 54 (1901); see also De and Roy Choudhury, *J. Indian Chem. Soc.*, **5**, 269 (1928).

(9) Hoggarth, *J. Chem. Soc.*, 1163 (1949).