4-*Aminosalicylaldehyde.*

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Bromination of 2-acetoxy-4-nitrotoluene gave a difficultly separable mixture of 2-acetoxy-4-nitrobenzyl bromide and 2-acetoxy-4-nitrobenzylidene dibromide. Treatment of the mixture with pyridine, however, afforded 2-hydroxy-4-nitrobenzylpyridinium bromide in good yield which was converted by the Kröhnke reaction into 4-nitrosalicylaldehyde. 4-Amino- and 4-acylamino-salicylaldehyde thiosemicarbazones were made from this in order to assess their antimycobacterial activites.

4-AMINO- AND 4-ACYLAMINO-SALICYLALDEHYDE THIOSEMICARBAZONES were required because they bear structural resemblance to 4-acetamidobenzaldehyde thiosemicarbazone and to 4-aminosalicylic acid, both of which compounds possess high activity against *Mycobacterium tuberculosis*. [Since the work described below was completed, Seymour, Drain, and Suddaby (B.P. 684,616) have described the preparation of 4-acetamidosalicylaldehyde thiosemicarbazone by a different method which does not, however, lend itself to large-scale application.]

The necessary intermediate, 4-nitrosalicylaldehyde, was obtained by Segusser and Calvin (*J. Amer. Chem. Soc.*, 1942, **64**, 825) in unspecified yield by bromination of 2-acetoxy-4-nitrotoluene and hydrolysis of the 2-acetoxy-4-nitrobenzylidene dibromide produced. Repetition of this procedure showed that the low yield (*ca.* 10%; cf. Libermann, Desnoes, and Hengl, *Compt. rend.*, 1951, **232**, 2027) was due to reluctance

of 2-acetoxy-4-nitrotoluene to undergo dibromination and difficulty of separation of mono- and di-bromination products. Examination of the reaction indicated that bromination of the substituted toluene with 2 mols. of bromine in boiling tetrachloroethane (see Table) produced the optimum amount of 2-acetoxy-4-nitrobenzyl bromide. Although this could not be isolated as such without great loss, treatment of the mixture with moist pyridine gave pure 2-hydroxy-4-nitrobenzylpyridinium bromide in acceptable yield; this was suitable for conversion into the required aldehyde by Kröhnke's reaction (*Ber.*, 1936, **69**, 2006; 1938, **71**, 2583; 1939, **72**, 440). Treatment of the quaternary salt with *NN*-dimethyl-*p*-nitrosoaniline and sodium hydroxide yielded the nitrone, 2-hydroxy-4-nitrobenzaldoxime *N*-*p*-dimethylaminophenyl ether, which without isolation was rapidly converted by dilute acid into 4-nitrosalicylaldehyde. Reduction of the thiosemicarbazone with hydrogen or ammonium sulphide yielded 4-aminosalicylaldehyde thiosemicarbazone; this, on treatment with acyl anhydrides or halides, gave the desired 4-acylaminosalicylaldehyde thiosemicarbazones.

Chemotherapeutic Properties.—Mice (20 g.), in groups of ten, each mouse infected with *M. tuberculosis* (Ravenel strain; 1 million organisms per mouse) and each fed with 75 or 150 mg./kg. doses daily of (i) 4-amino- (LD50 2·6 g./kg.), (ii) 4-acetamido- (LD50 3·0 g./kg.), (iii) 4-benzamido- (LD50 3·5 g./kg.), and (iv) 4-hexanamido-salicylaldehyde thiosemicarbazone (LD50 3·1 g./kg.) failed to survive for 25 days. The untreated controls died in 14—20 days; infected mice given 75 mg./kg. of 4-acetamidobenzaldehyde thiosemicarbazone (LD50 0·8 g./kg.) or 1250 mg./kg. of 4-aminosalicylic acid (LD50 4·0 g./kg.) daily survived for 35 days.

Experimental

2-Acetoxy-4-nitrobenzyl Bromide.—2-Methyl-5-nitrophenol (100 g.) was slowly added to stirred acetic anhydride (100 c.c.), and the mixture heated on the water-bath for 1 hr., cooled, and then poured into ice-water (600 c.c.). After ½ hour's stirring the crude 2-acetoxy-4-nitrotoluene (116 g.) was collected, washed, and dried at 40°/10 mm.; this had m. p. 70—74° and was pure enough for use. Dry bromine (23 g., 0·145 mol.) was added during 4 hr. to a refluxing solution of 2-acetoxy-4-nitrotoluene (28 g., 0·14 mol.) in dry carbon tetrachloride (400 c.c.), and the solution then refluxed for a further 24 hr. Solvent was removed and the residue crystallised from dried *n*-butanol; *2-acetoxy-4-nitrobenzyl bromide* (10 g., 26%) separated in plates, m. p. 79° (Found: Br, 29·1. $C_9H_8O_4NBr$ requires Br, 29·2%). Attempts to convert this into 4-nitrosalicylaldehyde by the Sommelet reaction were not very successful. Thus, hexamine (7 g.) was added rapidly to a refluxing solution of the foregoing bromide (10 g.) in acetic acid (25 c.c.). Refluxing was continued for a further ¼ hr. and the clear solution distilled in steam; 4-nitrosalicylaldehyde (0·5 g., 7%) came over in pale yellow plates, m. p. 132°. The method was abandoned in favour of the following.

2-Hydroxy-4-nitrobenzylpyridinium Bromide.—A solution of dry bromine (128 g., 0·8 mol.) in redistilled tetrachloroethane (40 c.c.) was added during 4 hr. to a refluxing solution of 2-acetoxy-4-nitrotoluene (80 g., 0·4 mol.) and iodine (0·2 g.) in tetrachloroethane (160 c.c.) in a quartz flask irradiated by a 500-watt ultra-violet lamp emitting in the 3000—3600 Å range. Refluxing was continued for a further 2 hr. by which time evolution of hydrogen bromide had ceased; solvent was pumped off and the residual oil (A) dissolved in 95% alcohol (160 c.c.). Pyridine (50 c.c.) was added with shaking, whereupon the mixture developed sufficient heat to effect refluxing of the alcohol and simultaneous separation of *2-hydroxy-4-nitrobenzylpyridinium bromide*. After storage overnight at room temperature, the crystalline precipitate (88 g., 71%; m. p. 264—266°) was collected, washed with a small amount of 90% alcohol, and dried; a sample separated from dilute alcohol in needles, m. p. 268° (Found: N, 9·2; Br, 25·9. $C_{12}H_{11}O_3N_2Br$ requires N, 9·0; Br, 25·7%). Use of a Pyrex flask caused a slight diminution in yield; in reactions carried out without ultra-violet illumination the yield was 63%. In one experiment the dark oil (A) resulting from the foregoing bromination was diluted with tetrachloroethane (400 c.c.), and this solution mixed with dry pyridine (100 c.c.) and heated on the water-bath for 1 hr.; on cooling, *2-acetoxy-4-nitrobenzylpyridinium bromide*, m. p. 230°, separated (Found: N, 8·1; Br, 22·9. $C_{14}H_{13}O_4N_2Br$ requires N, 7·9; Br, 22·6%). The same compound was obtained by refluxing 2-hydroxy-4-nitrobenzylpyridinium bromide with four times its weight of acetic anhydride for a short time.

The action of bromine under varying conditions upon 2-acetoxy-4-nitrotoluene is shown in

the annexed Table. The last column gives the amount of 2-acetoxy-4-nitrobenzyl bromide formed, as assessed by the yield of 2-hydroxy-4-nitrobenzylpyridinium bromide obtained by treatment with moist pyridine.

Bromination of 2-acetoxy-4-nitrotoluene with bromine.

Bromine (mol.)	Addition time (hr.)	Reflux time after addition (hr.)	Solvent	Yield (%)
1	5	12	CCl_4	11
4	9	12	,,	52
1	2·5	20	$C_2H_2Cl_4$	23
1·5	0·5	4	,,	39
2	3·5	5	,,	71
2	4·0	6	,,	70
3	2·5	4	,,	52

Bromination of 2-Acetoxy-4-nitrotoluene with N-*Bromosuccinimide and* N-*Bromophthalimide*.—(i) A mixture of 2-acetoxy-4-nitrotoluene (9·8 g., 0·05 mol.), *N*-bromosuccinimide (10 g., 0·056 mol.), benzoyl peroxide (0·2 g.), and tetrachloroethane (15 c.c.) was gently heated until a vigorous reaction set in; when this had subsided the mixture was refluxed for ½ hr. and kept overnight. The succinimide was filtered off, the solvent pumped off, and the residual oil refluxed for 1 hr. with 95% alcohol (20 c.c.) and pyridine (8 c.c.); 2-hydroxy-4-nitrobenzylpyridinium bromide (2·2 g., 15%) was obtained.

(ii) 2-Acetoxy-4-nitrotoluene (19·8 g., 0·1 mol.), *N*-bromophthalimide (24 g., 0·105 mol.), and tetrachloroethane (60 c.c.) were heated on the water-bath for 4 hr. and then the mixture refluxed for 4 hr. In the morning the precipitated phthalimide (9·9 g.) was removed and the filtrate treated as above described; the yield was 3 g. (24%).

4-*Nitrosalicylaldehyde*.—A suspension of 2-hydroxy-4-nitrobenzylpyridinium bromide (62·4 g., 0·2 mol.) and freshly prepared *NN*-dimethyl-*p*-nitrosoaniline (30 g., 0·2 mol.) in 96% alcohol (320 c.c.) was rapidly stirred at room temperature. N-Sodium hydroxide (400 c.c., 0·4 mol.) was added, stirring continued for 2 hr., and the dark red mixture set aside overnight (B); in the morning 5N-hydrochloric acid (800 c.c., 4 mol.) was added and the mixture stirred at room temperature for ½ hr. The brick-red precipitate of 4-nitrosalicylaldehyde (28 g., m. p. 130°) was collected, washed with water, and dissolved in boiling alcohol (150 c.c.), water (25 c.c.), and acetic acid (2 c.c.), and the filtered (charcoal) solution was diluted with boiling water (350 c.c.); 4-nitrosalicylaldehyde (20 g.) separated as glittering puce plates, m. p. 134° (Found: N, 8·6. Calc. for $C_7H_5O_4N$: N, 8·4%). The compound is soluble in a large volume of water; it is volatile in steam, separating in the distillate as long yellow needles of the same m. p.

A solution of the foregoing aldehyde (28 g.) in boiling alcohol (400 c.c.) was added slowly to a stirred solution of thiosemicarbazide (40 g.) in water (1200 c.c.) at 90°. After a further ½ hour's stirring and storage overnight, the yellow precipitate of 4-*nitrosalicylaldehyde thiosemicarbazone* (35 g.), m. p. 260°, was collected (Found: N, 23·4; S, 13·2. $C_8H_8O_3N_4S$ requires N, 23·3; S, 13·3%).

The following derivatives of 4-nitrosalicylaldehyde were prepared and crystallised from the solvent stated in parentheses: *Oxime* (alcohol), long yellow needles, m. p. 192° (Found: N, 15·4. $C_7H_6O_4N_2$ requires N, 15·4%); *hydrazone* (alcohol), orange plates, m. p. 176° (Found: N, 23·0. $C_7H_7O_3N_3$ requires N, 23·2%); 2 : 4-*dinitrophenylhydrazone* (dilute pyridine), orange-red clusters of needles, m. p. >310° (Found: N, 20·2. $C_{13}H_9O_7N_5$ requires N, 20·2%); p-*thiocyanatophenylhydrazone* (aqueous pyridine), puce needles, m. p. 226° (decomp.) (Found: N, 17·6; S, 10·2. $C_{14}H_{10}O_3N_4S$ requires N, 17·8; S, 10·2%); and 2-*hydroxyethylhydrazone* (aqueous alcohol), needles, m. p. 112° (Found: N, 18·6. $C_9H_{11}O_4N_3$ requires N, 18·7%).

2-*Hydroxy*-4-*nitrobenzaldoxime* N-p-*Dimethylaminophenyl Ether*.—When the foregoing red liquor (B) was filtered, this *nitrone* (64 g.) was obtained; it crystallised from ethyl acetate in glistening red plates, m. p. 254° (Found: N, 14·1. $C_{15}H_{15}O_4N_3$ requires N, 13·95%). Stirring this with 2·5N-hydrochloric acid (800 c.c.) at room temperature for ½ hr. gave 4-nitrosalicylaldehyde (20 g.).

4-*Aminosalicylaldehyde Thiosemicarbazone*.—Hydrogen sulphide was passed into a stirred suspension of 4-nitrosalicylaldehyde thiosemicarbazone (16·5 g.) in 96% alcohol (85 c.c.) and aqueous ammonia (85 c.c.; *d* 0·880) for 4 hr. The resulting solution was refluxed for 1 hr. with stirring and then kept at room temperature overnight. The crystalline precipitate was collected and dissolved in 0·25N-sodium hydroxide (420 c.c.), and the filtered solution (charcoal) stirred and acidified with 25% acetic acid; the *product* (9·3 g.) separated as a yellow microcrystalline powder, m. p. 222—226° (decomp.) (Found: N, 26·5; S, 15·2. $C_8H_{10}ON_4S$ requires N, 26·7;

S, 15·3%). Acylating agents react with the 4-amino-group before attacking the thiosemicarbazide grouping.

4-*Acetamidosalicylaldehyde Thiosemicarbazone*.—Acetic anhydride (5 c.c.) was added to a solution of the foregoing amino-compound (10·5 g.) in pyridine (100 c.c.), and the mixture set aside overnight. Crystallisation of the precipitate (6·8 g.) from acetic acid gave the pure *acetyl* compound, m. p. 260—264° (decomp.) (Found: N, 22·3; S, 12·5. $C_{10}H_{12}O_2N_4S$ requires N, 22·2; S, 12·7%).

The following derivatives were prepared (with F. P. JENKINS): 4-*benzamidosalicylaldehyde thiosemicarbazone* (from aqueous methanol), orange microcrystalline powder, m. p. 180—186° (decomp.) (Found: N, 18·1; S, 10·0. $C_{15}H_{14}O_2N_4S$ requires N, 17·8; S, 10·2%); 4-*salicylamidosalicylaldehyde thiosemicarbazone* (from aqueous ethanol), orange needles, sinters at 200—210° (decomp.) (Found: N, 16·8; S, 9·5. $C_{15}H_{14}O_3N_4S$ requires N, 16·9; S, 9·7%); 4-n-*hexanamidosalicylaldehyde thiosemicarbazone* (from aqueous alcohol), orange powder, m. p. 260—266° (decomp.) (Found: N, 18·7; S, 10·5. $C_{14}H_{20}O_2N_4S$ requires N, 18·2; S, 10·4%).

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