

NOTES

Some Heterocyclic Thiosemicarbazones

BY FLOYD E. ANDERSON, CHARLES J. DUCA AND JOHN V. SCUDI

Following the introduction of Tibione, (*p*-acetylaminobenzaldehyde thiosemicarbazone) by Domagk and co-workers,^{1,2,3} a number of investigators have attempted to improve upon this clinically

of furfuraldehyde, β -furylacrolein, thiophenealdehyde, tetrahydrothiapyran and isatin.

As part of an extensive program concerned with the chemotherapy of tuberculosis and leprosy we report here the synthesis of thiosemicarbazones of heterocyclic carbonyl compounds together with their *in vitro* activity against two pathogenic strains of mycobacteria.

TABLE I
HETEROCYCLIC THIOSEMICARBAZONES

Dubos' Tween-Albumin medium,¹ containing malachite green 1×10^{-6} concentration, was used for all *in vitro* tests. The compounds were dissolved in the Dubos medium (or other solvents when necessary) and using a twofold serial dilution method, final volumes were adjusted to 10 ml. with drug concentrations ranging from 64–0.25 mg. % *w./v.* The inoculum consisted of 0.1 ml. of a 14-day culture of tubercle bacilli in Dubos' medium, which was adjusted to an optical density of 2 at 530 μ as measured in the Lumetron colorimeter, model #400. Controls, including solvents when the compound was insoluble in water, were included in each test and all tests were run in duplicate. After 14 days incubation at 37°, the tubes were removed and examined. The highest concentration of drug in which no growth was observed visually was then taken as the minimal inhibitory concentration.

Carbonyl compounds used	M.p., °C.	Empirical formula	Nitrogen analyses %		Minimal bacteriostatic concentration mg. %	
			Calcd.	Found	H ₂ RV	B ₁
Furan-2-aldehyde ^a	152–154	C ₆ H ₇ NO ₃ S	24.8	24.5	0.5	0.25
2-Acetylfuran	142–144	C ₇ H ₉ N ₃ OS	22.9	22.5	1.0	0.25
2-Butyrylfuran	127–129	C ₉ H ₁₃ N ₃ OS	19.9	19.9	4.0	4.0
2,5-Dimethyl-3-acetylfuran	162–163	C ₉ H ₁₃ N ₃ OS	19.9	19.5	4.0	0.25
Furoin	144–145	C ₁₁ H ₁₁ N ₃ O ₃ S	15.8	15.4	8.0	16.0
Thiophene-2-aldehyde ^b	185–186	C ₆ H ₇ N ₃ S ₂	22.7	23.0	1.0	0.25
2-Acetylthiophene	148–149	C ₇ H ₉ N ₃ S ₂	21.1	20.8	4.0	4.0
2-Propionylthiophene ^c	127–128	C ₈ H ₁₁ N ₃ S ₂	19.7	19.9	1.0	0.06
2-Butyrylthiophene ^d	170–171	C ₉ H ₁₃ N ₃ S ₂	18.5	18.5	4.0	1.0
2,5-Dimethyl-3-acetylthiophene ^e	157	C ₉ H ₁₃ N ₃ S ₂	18.5	18.6	2.0	2.0
Pyridine-3-aldehyde ^f	213 dec.	C ₇ H ₈ N ₄ S	31.1	31.0	0.1	0.2
2-Acetylpyridine	158–160	C ₈ H ₁₀ N ₄ S	28.8	28.7	0.5	1.0
3-Acetylpyridine	217 dec.	C ₈ H ₁₀ N ₄ S	28.8	29.0	0.5	0.06
4-Acetylpyridine	218 dec.	C ₈ H ₁₀ N ₄ S	28.8	28.9	16.0	16.0
3-Butyrylpyridine	183–184	C ₁₀ H ₁₄ N ₄ S	25.2	24.9	4.0	12.0
3-Benzoylpyridine	175–176	C ₁₃ H ₁₂ N ₄ S	21.9	22.2	64.0	20.0
2,2,6,6-Tetramethyl-4-piperidone	190–193 dec.	C ₁₀ H ₂₀ N ₄ S	24.5	24.2	16.0	3.0
Pyrrole-2-aldehyde	195–197	C ₆ H ₈ N ₄ S	33.3	33.5	0.25	0.1
2-Butyrylpyrrole	171–172.5	C ₉ H ₁₄ N ₄ S	26.6	26.6	2.0	4.0
2,5-Dimethyl-3-acetylpyrrole	200 dec.	C ₉ H ₁₄ N ₄ S	26.6	27.2	2.0	0.25

^a Bernstein, *et al.*,⁶ reported the m.p. as 149–150°. ^b Bernstein, *et al.*,⁶ reported the m.p. as 186–187°. ^c Calcd.: S, 30.1. Found: S, 29.7. ^d Calcd.: S, 28.2. Found: S, 28.0. ^e Calcd.: S, 28.2. Found: 27.9. ^f C. Levaditi, A. Vaisman and A. Ray, *Compt. rend.*, **231**, 1174 (1950), have reported the m.p. as 227–230° dec.

important antimycobacterial agent. Stillman and Scott⁴ reported the thiosemicarbazone of 2-(5-nitrofurfuryl)-aldehyde. Hoggarth, *et al.*,⁵ reported the activity of the thiosemicarbazones of numerous substituted benzaldehydes and of two quinoline-carboxaldehydes. More recently Bernstein and associates⁶ described the synthesis of many thiosemicarbazones. Among these were derivatives

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Experimental

Thiosemicarbazones.—All thiosemicarbazones were prepared by interaction of the aldehyde or ketone with thiosemicarbazide in water or in 60% alcohol-water. Usually six to eight hours of heating at steam-bath temperatures was sufficient, although a reaction time of 80 hours was required to obtain adequate yields of the thiosemicarbazone of 3-benzoylpyridine. In general, thiosemicarbazide is more water-soluble than the thiosemicarbazones; consequently, recrystallization from water or trituration with boiling water was routinely employed prior to recrystallization from alcohol or 50% ethyl alcohol-water.

The general method of synthesis of the thiosemicarbazones is illustrated by the following example: A solution containing 5.0 g. (0.041 mole) of 4-acetylpyridine and 3.64 g. (0.040 mole) of thiosemicarbazide in 200 ml. of distilled water was heated under reflux for a period of seven hours. When the reaction mixture was allowed to cool slowly, a white crystalline product separated; weight 7.45 g. (96% yield). The finely ground product, extracted with 300 ml. of boiling water, was then recrystallized from ethyl alcohol; final yield 5.0 g., m.p. 219–222° dec. (All melting points are uncorrected.)

Intermediates.—The compounds 2-acetylthiophene, 2-propionylthiophene, 2-butyrylthiophene, 2-butyrylfuran and 2-acetylfuran were prepared according to Heid and Levine⁷ by interaction of thiophene (or furan) with the appropriate acid anhydride in the presence of boron trifluoride. Application of this method was extended to prepare 2,5-dimethyl-3-acetylthiophene,⁸ 2,5-dimethyl-3-acetylfuran⁹ and 2-butyrylpyrrole¹⁰ in yields of 50, 70 and 16.5%, respectively. The physical constants of these compounds were found to be in agreement with those reported in the literature. Treatment of 2,5-dimethyl-2-acetylfuran with excess aqueous ammonia in a sealed tube at 145° for four hours gave the corresponding pyrrole.⁹ Decarboxylation of the appropriate keto acids according to the procedure of Burrus and Powell¹¹ gave 2-acetylpyridine, 4-acetylpyridine, 3-acetylpyridine and 3-propionylpyridine. Application of this method was extended without modification to prepare 3-butyrylpyridine¹² in approximately 15% yields. Interaction of nicotinoyl chloride and benzene in the presence of aluminum chloride gave good yields of 3-benzoylpyridine.¹³ Pyridine-3-carboxaldehyde was prepared by the procedure of Panizzon.¹⁴ Thiophene-2-carboxaldehyde was purchased from the Arapahoe Chemical Co., Boulder, Colorado.

Acknowledgment.—We are indebted to David Regenbogen and Sydney Gister for the preparation of intermediates and to Ruth Becker for microanalytical data.

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Molar Refractions in the Binary System Acetone-Carbon Tetrachloride

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In connection with vapor-liquid equilibrium studies of the binary system acetone-carbon tetrachloride, both densities and refractive indices of known solutions covering the entire composition

TABLE I

MOLAR REFRACTIONS IN THE SYSTEM ACETONE-CARBON TETRACHLORIDE

$$[R]_D, \text{ obsd.} = \left(\frac{n^2 - 1}{n^2 + 2} \right) \times \left(\frac{x_1 M_1 + x_2 M_2}{d} \right); [R]_D, \text{ calcd.} = 16.167 + 10.280x_2$$

Mole fraction CCl ₄ , x_2	Molar refraction [R] _D obsd.	[R] _D calcd.	ΔR
1.000	26.447	(26.447)	
0.8978	24.426	25.396	0.030
.8027	24.461	24.419	.042
.6990	23.394	23.353	.041
.5993	22.371	22.328	.043
.4996	21.345	21.303	.042
.4005	20.309	20.284	.025
.3010	19.292	19.261	.031
.2004	18.250	18.227	.023
.1003	17.212	17.198	.014
.0000	16.167	(16.167)	

(1) M. W. Kellogg Co., Jersey City, N. J.

(2) General Electric Research Laboratory, Schenectady, N. Y.

range were made at 25°. In Table I are shown the molar refractions obtained from these data along with those calculated assuming a linear variation with composition.

In spite of the large positive deviations from ideal behavior indicated by the vapor-liquid equilibrium data, the deviations from additivity of the molar refractions, though varying with composition, never exceed 0.045 cc. This observation is in agreement with the more limited data (0-40 mole per cent. acetone) of Smyth, Engel and Wilson⁴ and with the unpublished results of Koenig-Gressman.⁵

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RECEIVED APRIL 13, 1951

The Failure of Crystalline Vitamin B₁₂ to Exchange with Co⁶⁰ in Acidic and Neutral Aqueous Solutions

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Because of the recent availability and interest in the growth-promoting characteristics and metabolism of vitamin B₁₂, it was decided to determine whether a radioactive form of the vitamin could be prepared for study by simple exchange with Co⁶⁰, over a period of several weeks. Fantes, Page, Parker and Smith¹ have shown that this exchange does not occur over a period of two hours at room temperature in 0.1 N acid or alkali, or by boiling for one hour at pH 7. However, the possibility of exchange on prolonged contact has not been examined.

The possibility of such an exchange was indicated by application of paper chromatography. Co⁶⁰SO₄ in solution with B₁₂ was observed to migrate with B₁₂, while a control spot of Co⁶⁰SO₄ did not migrate. To determine whether this is an actual exchange or simply a weak complexing or surface action, the following experiment was performed.

The approximate analysis of the cobalt content of B₁₂ is 4%.² An aqueous solution was prepared containing 0.1 mg./ml. crystalline Merck vitamin B₁₂. Approximately ten times the amount of cobalt present in the B₁₂ was added in the form of Co⁶⁰SO₄. The mixture was allowed to stand at room temperature for five weeks at a pH of about 2. This B₁₂ cobalt mixture exhibited growth stimulation on *Lactobacillus leichmannii* (ATC 4797). Model experiments had indicated that B₁₂ could be salted out of water and into *n*-butanol by the addition of solid ammonium sulfate. Cobalt under these conditions remained in the aqueous phase. Repeated application of the salting-out procedure to the radioactive mixture resulted in a final butanol phase, strongly colored by the vitamin but exhibiting no radioactivity. This procedure was repeated with a neutral solution of B₁₂ and Co⁶⁰SO₄ with the same negative results.

The experimental results are recorded in Table I. A thin-window counting tube was employed in the measurements. Accordingly, under the above conditions, vitamin B₁₂ does not exchange with radioactive cobalt, and the conclusions of Fantes, *et al.*, concerning the strong coordinate

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