

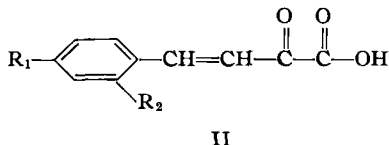
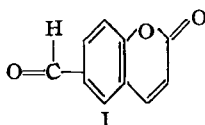
commonly found in the manufacturing laboratory: the Premier colloid mill and the Eppenbach Homo-Mixer. Simple agitation in a screw-top jar was used as an intermediate procedure. Duplicate experiments (using these pieces of equipment) show the official method of extraction of digitalis (using the percolator) to be superior to methods using the other pieces of equipment tested.

Antitubercular Activity of Some Aromatic Aldehyde and Ketone Derivatives

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The semicarbazones, para-nitrobenzoyl hydrazones and isonicotinyl hydrazones or the unsubstituted and three substituted 4-phenyl-2-oxo-3-butenic acid and 6-formyl-1,2-benzpyrone were prepared and the toxicities and *in vitro* antitubercular activities measured. Three isonicotinyl hydrazones show the same order of antitubercular activity as isonicotinic acid hydrazide.

THE WELL KNOWN antitubercular activity of thiosemicarbazones, *p*-nitrobenzoyl hydrazones, and isonicotinyl hydrazones of a number of substituted and α,β -unsaturated aromatic aldehydes (1, 6) and ketones (2) made it of interest to prepare the corresponding derivatives of 6-formyl-1,2-benzpyrone (I) and of 4-phenyl-2-oxo-3-butenic acid (II; $R_1, R_2 = H$), the latter possessing the additional advantage of affording water-soluble salts.



In view of the interesting antitubercular activity of the isonicotinyl hydrazone of II, the corresponding *o*-chloro, *o*-methoxy, and *p*-methoxy substituted derivatives of II were prepared for comparison and are described in Table I.

In vitro antitubercular activity was determined against *Mycobacterium tuberculosis* var. *hominus* strain H 37 RV by the serial dilution technique, using the modified Dubos medium (3). The results are shown in Table II.

Acute toxicity of the compounds was determined

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using intraperitoneal injections of suspensions in 6% acacia in Webster strain mice of both sexes (average weight 20 Gm.). The results are given in Table III.

EXPERIMENTAL¹

6-Formyl-1,2-benzpyrone.—Prepared by the method of Sen and Chakravarti (4), the compound melted at 189° (lit. m.p. 187–189°) (4).

6-Formyl-1,2-benzpyrone Thiosemicarbazone.—A mixture of 0.8 Gm. of 6-formyl-1,2-benzpyrone in 35 ml. of 80% ethanol, 0.45 Gm. of thiosemicarbazide, and 1 Gm. of sodium acetate was refluxed on a steam cone for 1 hour. The reaction product was recrystallized from dilute alcohol. One gram (85% yield) of a cream-colored crystalline product was obtained. The compound sublimates without melting at 300°.

Anal.—Calcd. for $C_{11}H_9N_3O_2S$: N, 17.0. Found: N, 16.79.

6-Formyl-1,2-benzpyrone *p*-Nitrobenzoyl Hydrazone.—A mixture of 0.9 Gm. of 6-formyl-1,2-benzpyrone in 35 ml. of 80% ethanol and 0.9 Gm. of *p*-nitrobenzoyl hydrazide in 20 ml. of ethanol was refluxed for 1.5 hours on a steam bath. The reaction product was recrystallized from 30% alcohol from which 1.5 Gm. (90% yield) of lemon-yellow crystals was obtained, m.p. 314–315°.

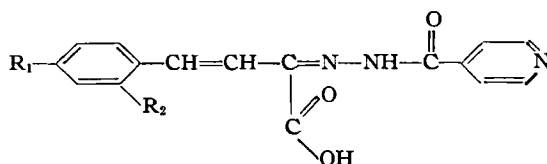
Anal.—Calcd. for $C_{17}H_{11}N_3O_5$: N, 12.46. Found: 12.7.

6-Formyl-1,2-benzpyrone Isonicotinyl Hydrazone.—A 0.7-Gm. quantity of isonicotinic acid hydrazide in 10 ml. of distilled water was added to a solution of 0.9 Gm. of 6-formyl-1,2-benzpyrone in 35 ml. of 80% ethanol. The mixture was heated on a steam bath; white crystals started separating immediately. The crude product was recrystallized from water. The product weighed 1.4 Gm. (92% yield) and melted at 284–285°.

Anal.—Calcd. for $C_{16}H_{10}N_3O$: N, 14.33. Found: N, 14.53.

¹ Microanalysis of the compounds reported was carried out by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

TABLE I.—UNSUBSTITUTED AND SUBSTITUTED 4-PHENYL-2-OXO-3-BUTENOIC ACID ISONICOTINYL HYDRAZONES



R ₁	R ₂	Yield, %	M.p., °C.	Formula	Found, N %	Calcd., N %
H	H	90	308	C ₁₆ H ₁₃ N ₃ O ₂	14.50	14.23
H	Me—O	90	185.5	C ₁₇ H ₁₅ N ₃ O ₄	12.89	12.96
Me—O	H	92	201–202	C ₁₇ H ₁₅ N ₃ O ₄	12.79	12.96
H	Cl	85	190 to 190.5	C ₁₆ H ₁₂ N ₃ O ₃ Cl	12.81	12.74

TABLE II.—ANTITUBERCULAR ACTIVITY

Compound	Required for Inhibition of Growth, min. mol. concn.
4-Phenyl-2-oxo-3-butenoic acid	
-thiosemicarbazone	10 ⁻⁴
- <i>p</i> -nitrobenzoyl hydrazone	10 ⁻³
-isonicotinyl hydrazone	10 ⁻⁵
4-(<i>p</i> -Methoxyphenyl)-2-oxo-3-butenoic acid	
-thiosemicarbazone	10 ⁻⁴
-isonicotinyl hydrazone	10 ⁻⁵
- <i>p</i> -nitrobenzoyl hydrazone	10 ⁻³
4-(<i>o</i> -Methoxyphenyl)-2-oxo-3-butenoic acid	
-isonicotinyl hydrazone	10 ⁻⁵
4- <i>o</i> -Chlorophenyl-2-oxo-3-butenoic acid	
-isonicotinyl hydrazone	10 ⁻⁵
6-Formyl-1,2-benzpyrone	
-semicarbazone	10 ⁻⁴
- <i>p</i> -nitrobenzoyl hydrazone	10 ⁻³
-isonicotinyl hydrazone	10 ⁻⁵
Isonicotinic acid hydrazide	10 ⁻⁵

TABLE III.—ACUTE TOXICITY IN MICE (INTRAPERITONEALLY)

Compound	LD ₅₀ , mg./Kg.
6-Formyl-1,2-benzpyrone	
-thiosemicarbazone	350
- <i>p</i> -nitrobenzoyl hydrazone	>350
-isonicotinyl hydrazone	>500
4-Phenyl-2-oxo-3-butenoic acid	
-isonicotinyl hydrazone ^a	>750

^a The LD₅₀ of all compounds listed in Table I was greater than 750.

4-Phenyl-2-oxo-3-butenoic Acid Thiosemicarbazone.—Prepared as described above, this was obtained in 75% yield as yellow needles, m.p. 191° dec.

Anal.—Calcd. for C₁₁H₁₁O₂N₂S: N, 16.87. Found: N, 17.02.

4-Phenyl-2-oxo-3-butenoic Acid *p*-Nitrobenzoyl Hydrazone.—By the method described, this formed greenish-yellow needles in 70% yield, m.p. 231°.

Anal.—Calcd. for C₁₇H₁₃O₅N₃: N, 12.36. Found: N, 12.49.

4-Phenyl-2-oxo-3-butenoic Acid Isonicotinyl Hydrazone.—Prepared as described under 6-Formyl-1,2-benzpyrone Isonicotinyl Hydrazone. The compound recrystallized from 33% alcohol was obtained in 90% yield as light cream-colored needles, m.p. 308°.

Anal.—Calcd. for C₁₆H₁₃N₃O₃: N, 14.23. Found: N, 14.50.

Three Phenyl Substituted Isonicotinyl Hydrazones of 4-Phenyl-2-oxo-3-butenoic Acid.—These were prepared following the same procedure and are listed under Table I.

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4-Phenyl-2-oxo-3-butenoic Acid.—Prepared by the method of Erlenmeyer (5), the compound melted at 50° (lit. m.p. 53°) (5).

Anal.—Calcd. for C₁₀H₈O₃: C, 68.18; H, 4.54. Found: C, 68.33; H, 4.72.

4-*o*-Methoxyphenyl-2-oxo-3-butenoic Acid.—Prepared by the same procedure, this acid was obtained as light-orange needles in 50% yield, m.p. 133–134°.

Anal.—Calcd. for C₁₇H₁₅N₃O₄: N, 12.96. Found: 12.89.

4-*p*-Methoxyphenyl-2-oxo-3-butenoic Acid.—By the method described, this formed yellow needles in 62% yield, m.p. 126° dec. (lit. m.p. 131°) (7).

4-*o*-Chlorophenyl-2-oxo-3-butenoic Acid.—Prepared as described above, this acid was obtained in 62% yield as light-yellow crystals, m.p. 195° dec.

Anal.—Calcd. for C₁₆H₁₂N₃O₃Cl: N, 12.74. Found: N, 12.81.