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Antitubercular Activity of Substituted 5-Oxo-1-thiocarbamoyl-3-pyrazoline-4-alkanoic Acid Derivatives

ARTHUR A. SANTILLI [x], DONG H. KIM, and FRANCIS J. GREGORY

Abstract □ Several novel pyrazolin-5-ones prepared by the cyclization of variously substituted thiosemicarbazone derivatives of ethyl formylsuccinate, ethyl acetylsuccinate, and ethyl acetylglutarate were tested for antitubercular activity against *Mycobacterium tuberculosis*, human type, strain H37Rv, by a tube dilution technique. Minimum inhibitory concentrations (MIC) for these derivatives ranged from 0.05 to 100 μg/ml. The most active compound was ethyl 3-methyl-1-methylthiocarbamoyl-5-oxo-3-pyrazoline-4-acetate (MIC = 0.05–0.1 μg/ml).

Keyphrases □ 5-Oxo-1-thiocarbamoyl-3-pyrazoline-4-alkanoic acid derivatives—synthesis, antitubercular activity □ Pyrazolin-5-one derivatives—synthesis, antitubercular activity □ Antitubercular activity—synthesis and screening of substituted 5-oxo-1-thiocarbamoyl-3-pyrazoline-4-alkanoic acid derivatives

Various classes of thiosemicarbazones have been of interest to clinicians for their therapeutic value as antitubercular, antiviral, antileprotic, and antifungal agents (1–3). Recently, the synthesis of a group of variously substituted 5-oxo-1-thiocarbamoyl-3-pyrazoline-4-alkanoic acid derivatives (II*a*–II*l*), which were prepared by the cyclization of open-chain thiosemicarbazones (I) of diethyl formylsuccinate, diethyl acetylsuccinate, and diethyl acetylglutarate, was described (4). Spectral evidence indicates that these compounds also exist in their tautomeric 5-hydroxypyrazole form (II′*a*–II′*l*). Several of these derivatives were tested for their overall therapeutic value as anti-infective agents and were found to possess substantial antitubercular activity against *Mycobacterium tuberculosis*, human type. A summary of the biological test results in relation to chemical structure is reported here.

EXPERIMENTAL[1]

The open-chain thiosemicarbazones (I) were prepared by heating ethyl formylsuccinate, ethyl acetylsuccinate, or ethyl acetylglutarate with 1 equivalent of a suitably substituted thiosemicarbazide (Scheme I). Ring closures of these derivatives were carried out by warming the various thiosemicarbazones in ammonium hydroxide solution followed by acidification; the title compounds were obtained (Table I). The esters were readily converted to the corresponding acids by hydrolysis using sodium hydroxide solution.

Synthesis—The following example typifies the method used to prepare the title compounds. Further preparative details as well as the chemistry and spectral analyses for other compounds were previously published (4).

Diethyl Acetylsuccinate Thiosemicarbazone (I)—A mixture of 10.8 g of diethyl acetylsuccinate and 4.56 g of thiosemicarbazide in 250 ml of ethanol was heated under reflux for 17 hr. The ethanol solution was cooled in ice, and cyclohexane was added to precipitate the product. The product amounted to 13.1 g, mp 98–102°. The analytical sample (mp 98–100°) was obtained by recrystallization from ethanol–cyclohexane; IR (KBr): 2.90, 3.00, 3.12 (NH), 5.72, and 5.79 (ester C=O) μm.

Anal.—Calc. for $C_{11}H_{19}N_3O_4S$: C, 45.66; H, 6.62; N, 14.52; S, 11.08. Found: C, 45.64; H, 6.58; N, 14.65; S, 11.42.

Ethyl 3-Methyl-5-oxo-1-thiocarbamoyl-3-pyrazoline-4-acetate (IIa)—A mixture of 10 g of diethyl acetylsuccinate thiosemicarbazone and 250 ml of concentrated ammonium hydroxide solution was heated on a steam bath for approximately 30 min, at which

[1] Melting points were determined with a Thomas-Hoover capillary melting-point apparatus and are uncorrected. The IR spectra were determined in potassium bromide disks using a Perkin-Elmer model 21 spectrophotometer.

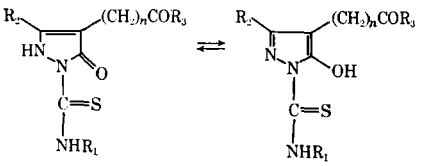
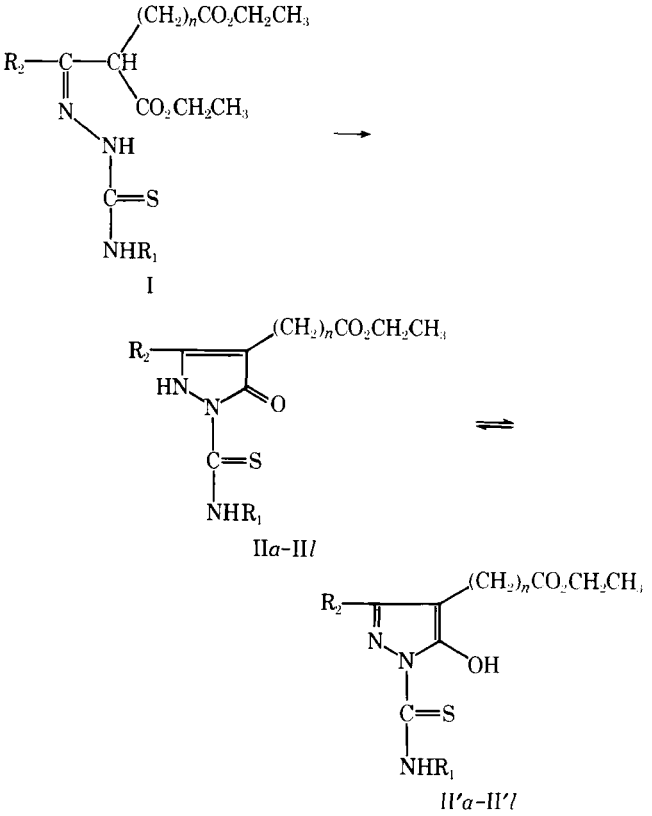


Table I—Substituted 5-Oxo-1-thiocarbamoyl-3-pyrazoline-4-alkanoic Acid Derivatives and Their *In Vitro* Activity *versus M. tuberculosis*, Human Type

Compound	n	R_1	R_2	R_3	Melting Point	Formula	Activity *versus* H37Rv, MIC, μg/ml
II*a*	1	H	CH_3	OCH_2CH_3	146–148°	$C_9H_{13}N_3O_3S$	0.5
II*b*	1	H	CH_3	OH	178–179°	$C_7H_9N_3O_3S$	50
II*c*	1	CH_3	CH_3	OCH_2CH_3	151–153°	$C_{10}H_{15}N_3O_3S$	0.05–0.1
II*d*	1	CH_3	CH_3	NH_2	181–183°	$C_8H_{12}N_4O_2S$	5.0
II*e*	1	CH_3	CH_3	OH	180–182°	$C_8H_{11}N_3O_3S$	50
II*f*	1	CH_3	H	OCH_2CH_3	149–151°	$C_9H_{13}N_3O_3S$	0.1
II*g*	1	CH_3	H	OH	192–194°	$C_7H_9N_3O_3S$	50
II*h*	1	H	H	OCH_2CH_3	141–142°	$C_8H_{11}N_3O_3S$	100
II*i*	2	H	CH_3	OCH_2CH_3	149–150°	$C_{10}H_{15}N_3O_3S$	0.5
II*j*	2	H	CH_3	OH	186–187°	$C_8H_{11}N_3O_3S$	50
II*k*	2	CH_2CH_3	CH_3	OCH_2CH_3	72–74°	$C_{12}H_{19}N_3O_3S$	—
II*l*	2	CH_2CH_3	CH_3	OH	152–153° dec.	$C_{10}H_{15}N_3O_3S$	10



Scheme I

time the reaction mixture was filtered free of some undissolved particles. The solution was cooled in ice and acidified with concentrated hydrochloric acid. The precipitate which formed amounted to 3 g. The analytical sample (mp 146–148°) was obtained by recrystallization from ethanol; IR (KBr): 3.08 (NH), 5.72 (ester C=O), and 6.02 (lactam C=O) μm.

Anal.—Calc. for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.38; N, 17.27; S, 13.18. Found: C, 44.44; H, 5.40; N, 17.64; S, 13.43.

Biological—The compounds described here were tested for their bacteriostatic activity against *M. tuberculosis*, human type, strain H37Rv, by a tube dilution technique. The medium employed was Dubos oleic acid liquid medium. Stock cultures were maintained on Dorset egg agar. Stock solutions of 1000 μg/ml of drug were prepared in distilled water or dimethylacetamide, and aqueous solutions were sterilized by passage through a Swinny filter. One-milliliter quantities of each dilution were incorporated into 9 ml of medium to give final concentration values of 0.01–100 μg/ml. Tubes were seeded with 0.1 ml of standardized bacterial suspension and incubated for 2 weeks at 37°. The minimum inhibitory concentration (MIC), expressed in micrograms per milliliter, is the least amount of material required for complete inhibition of growth of *M. tuberculosis*.

RESULTS AND DISCUSSION

Compound IIc (Table I) showed the highest order of activity of the compounds tested (MIC ≈ 0.05–0.1 μg/ml). Activity diminished considerably when this compound was tested as the free acid (II*e*) or as a carboxamide (II*d*). Replacement of the methylthiocarbamoyl group by a thiocarbamoyl group (II*a*) also resulted in diminished activity. Replacement of the 3-methyl group in IIc by a hydrogen afforded II*f*, which showed a high order of activity similar to that of IIc.

An activity comparison for II*a* and II*i* indicates that no gain in potency results when the alkanoic acid chain is extended by one methylene group. A comparison of IIc with 3-methyl-5-oxo-3-pyrazoline-4-acetate (4) (MIC = 0.5 μg/ml) indicates that the methylthiocarbamoyl group must contribute significantly toward activity in this series since potency diminished considerably in its absence. Similarly, removal of the alkanoic acid group appears to have a deleterious effect on activity. This supposition is based on the fact that a sharp decline in potency was observed when 3-methyl-5-oxo-1-methylthiocarbamoyl-3-pyrazoline (4) (MIC = 1 μg/ml) was compared with IIc. The clinical agent isoniazid was used as the standard for comparison. When tested in this assay, its MIC was 0.005–0.01 μg/ml.

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