

cule as oxirane oxygen. Details will be published at some later date.

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### 3-Indolecarboxaldehyde Thiosemicarbazone, a New Antitubercular Compound<sup>1</sup>

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The inhibition of growth of *Mycobacterium tuberculosis* by many thiosemicarbazones has been reported.<sup>2a,b,3</sup> 3-Indolecarboxaldehyde thiosemicarbazone has been shown to have high bacteriostatic activity *in vitro* and to suppress tuberculosist in mice after injection of virulent tubercle bacilli. Because of these properties the synthesis of this new thiosemicarbazone is described.

**3-Indolecarboxaldehyde Thiosemicarbazone.**—Thiosemicarbazide 8.2 g. (0.09 mole), dissolved in 100 ml. of warm 30% acetic acid was added to a solution of 12.3 g. (0.085 mole) of 3-indolecarboxaldehyde<sup>4,5</sup> in 200 ml. of methanol and the resulting mixture refluxed for 2 hours. After cooling, the precipitate was collected by filtration, washed with cold water and air-dried. The crude product was purified by recrystallization from methanol to give light yellow crystals of 3-indolecarboxaldehyde thiosemicarbazone, m.p. 230–232° dec.

*Anal.* Calcd. for C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>S: N, 25.67. Found: N, 25.48.

**Biological Properties.**—The following organisms were not inhibited by 100 micrograms of 3-indolecarboxaldehyde thiosemicarbazone: *Diplococcus pneumoniae*, *Streptococcus hemolyticus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Micrococcus pyogenes* var. *aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Shigella dysenteriae*, *Corynebacterium diphtheriae*, *Aerobacter aerogenes*, *Mycobacterium phlei*, *M. smegmatis* and *Mycobacterium 607*.

*M. tuberculosis* var. *hominis*, strains H37R<sub>v</sub>, H37R<sub>a</sub> and T81760 were inhibited by 6.2, 0.8 and 3.2 micrograms of the chemical, respectively.

When injected intraperitoneally as a suspension, 50 mg. was lethal for 20-g. white mice while 25 mg. was tolerated. Four-and-one-half mg. injected daily on seven successive days was tolerated and this dose was used for a preliminary protection test. For this test, 1.0 mg. of *M. tuberculosis*, H37R<sub>v</sub> was injected intravenously into eight mice and the following day, 4.5 mg. of the 3-indolecarboxaldehyde was injected subcutaneously. Daily injections of the same dose of chemical were made for 17 days. Nineteen days after the last injection, seven of the eight treated mice were still alive while of the 10 control mice infected at the same time, but which did not receive the chemical, only three survived.

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(1) Journal article No. 1515, Michigan Agricultural Experiment Station.

(2) (a) G. Domagk, R. Behnisch, F. Mietzsch and H. Schmidt, *Naturwissenschaften*, **33**, 315 (1946); (b) G. Domagk, *Schweiz. Z. Path. u. Bakt.*, **12**, 575 (1949).

(3) G. Domagk, *Beitr. Klin. Tuberk.*, **103**, 603 (1950).

(4) F. T. Tyson and J. T. Shaw, *THIS JOURNAL*, **74**, 2273 (1952).

(5) E. Campaigne and W. L. Archer, *ibid.*, **76**, 989 (1953).

### The Keto Acids of the Tulip (*Tulipa gesneriana*) with Special Reference to the Keto Analog of $\gamma$ -Methyleneglutamic Acid

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Our interest in the keto acids of plants, because of their importance in the understanding of nitrogen metabolism, has prompted the development of a general method for their identification and quantitative determination. This method, which will be published in due course (Towers, Thompson and Steward) depends upon the following procedures.

a. The plant material is killed and the keto compounds fixed with an alcoholic solution of either 2,4-dinitrophenylhydrazine or 1,1-diphenylhydrazine.

b. The keto-acid hydrazones are separated from the amino acids and neutral carbonyl compounds by extraction into ethyl acetate and subsequent extraction of the ethyl acetate solution with dilute sodium carbonate.

c. Specific 2,4-dinitrophenylhydrazones are recognized by chromatography on paper.

d. The hydrazones are converted to the corresponding amino compounds by catalytic hydrogenolysis.

e. The amino compounds so produced are recognized by two-directional chromatography on paper and treatment of the chromatograms with ninhydrin. Thus the keto-acids may be recognized by drawing upon the extensive information, which is now available, on the chromatography of the amino compounds on paper and by utilizing the sensitivity of the ninhydrin method for their detection.

The recent recognition of  $\gamma$ -methyleneglutamine and of  $\gamma$ -methyleneglutamic acid as constituents of the peanut plant<sup>2</sup> and the proof that these substances are identical with nitrogenous compounds earlier recognized in the tulip plant<sup>3,4</sup> gives rise to interesting possibilities.

Therefore, in making the first extensive examination of the ketoacids of the tulip plant, special attention was paid to the recognition and identification of the keto analog of  $\gamma$ -methyleneglutamic acid. Work has been done on the keto compounds present in the tissue of the resting tulip bulb (in which the  $\gamma$ -methyleneglutamine was first recognized) and also of the green foliage leaves in which it is present in larger amount.

The 2,4-dinitrophenylhydrazones from the tulip bulb yielded on hydrogenolysis the following amino compounds: glycine, alanine, aspartic acid, glutamic acid and valine. In addition to these known amino acids, hydrogenolysis also yielded amino compounds whose identity still remains unknown. Therefore, one could infer that the following keto compounds, corresponding to this list of amino acids, are present as constituents of the tulip plant: glyoxylic acid, pyruvic acid, oxaloacetic acid,  $\alpha$ -ketoglutaric acid and unknown keto compounds.

(1) Lalor Foundation, Pre-doctoral Fellow at Cornell University.

(2) J. Done and L. Fowden, *Biochem. J.*, **49**, Proc. XX (1951).

(3) F. C. Steward and J. F. Thompson, *Ann. Rev. Plant Physiol.*, **2**, 233 (1950).

(4) R. Zacharias, J. K. Pollard and F. C. Steward, *THIS JOURNAL*, **76**, 1961 (1954).