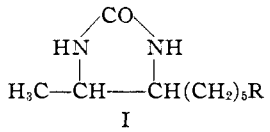
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

The Synthesis and Biological Activity of 4-Methyl-5-(ϵ-sulfoamyl)-2-imidazolidone, a Sulfonic Acid Analog of Desthiobiotin

BY ROBERT DUSCHINSKY AND SAUL H. RUBIN

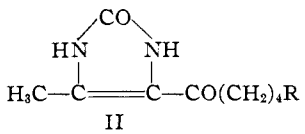
Replacement of carbonyl by sulfonyl groups in metabolites sometimes results in substances of antagonistic biological behavior.[1] In this respect the study of a sulfonic acid analog (I, R = SO_3H)



I

of desthiobiotin (I, R = CO_2H) appeared of interest. The substance was therefore synthesized and tested microbiologically.[2]

The general methods involved in the synthesis have been described previously.[3] 4-Methyl-2-imidazolone and δ-bromovaleryl chloride were submitted to a Friedel-Crafts condensation. The bromoketone obtained (II, R = Br) was converted



II

into the sodium sulfonate (II, R = SO_3Na) which was reduced catalytically to give the sodium salt of 4-methyl-5-(δ-sulfoamyl)-2-imidazolidone (I, R = SO_3Na). As in the completely analogous step in the synthesis of desthiobiotin,[3] two moles of hydrogen corresponding to the reduction of the keto group were absorbed rapidly, while the third mole corresponding to the hydrogenation of the double bond was taken up much more slowly. In view of the similarities of method and behavior, it is assumed that, as in the desthiobiotin synthesis, *cis*-addition at the double bond takes place.

The growth-promoting or growth-inhibiting properties of the compound for *Saccharomyces cerevisiae* 139 and *Lactobacillus casei* were tested by methods which have been previously reported.[4]

The sulfo compound did not support growth in biotin-free media. For *S. cerevisiae* it proved to be an antibiotin at a molar inhibition ratio of about 300,000. When *d,l*-O-heterobiotin was substituted for biotin in the medium, the ratio was found to be 17,000. This result presents additional evidence for the reported greater resistance of biotin toward inhibitors as compared to its oxygen analog.[5] When *d,l*-desthiobiotin was substituted for biotin, the growth of *S. cerevisiae* was inhibited at a molar ratio of 40,000. With *L. casei* no inhibition of growth was noticed at a molar ratio of more than 5,000,000 in media supplemented with *d*-biotin or *d,l*-O-heterobiotin.

Experimental[6]

4-Methyl-5-(δ-bromovaleryl)-2-imidazolone (II, R = Br).—δ-Bromovaleric acid was prepared in good yield by the procedure of Hunsdiecker and Hunsdiecker[7] by the reaction of methyl silver adipate with bromine and saponification of the resulting ester with a mixture of acetic and hydrobromic acids. The product was converted into the acid chloride[8] by means of thionyl chloride. The δ-bromovaleryl chloride (26.7 g.) was added to a suspension of 13.1 g. of 4-methyl-2-imidazolone[3] in 130 cc. of nitrobenzene. Aluminum chloride(44 g.) was added gradually with continuous stirring and occasional cooling. After heating the mixture for four and one-half hours in a bath at 60°, the evolved hydrochloric gas being driven off with a slow stream of nitrogen, it was poured on 200 g. of ice. Stirring and addition of 200 cc. of ether produced crystals, which were washed well with water and ether. The yield was 16.2 g. (43%), m. p. 199–201° (dec.). Recrystallization from 95% ethanol gave rectangular plates melting at 206–207° (dec.).

Anal. Calcd. for $C_9H_{13}O_2N_2Br$: C, 41.39; H, 5.02; Br, 30.61. Found: C, 41.92; H, 5.20; Br, 30.33.

Sodium Salt of 4-Methyl-5-(δ-sulfovaleryl)-2-imidazolone (II, R = SO_3Na).—A mixture of 2.61 g. of the bromoketone, 1.38 g. (1.1 moles) of sodium sulfite and 8.5 cc. of water was refluxed for two hours. The solution was evaporated to dryness and the residue boiled with 22 cc. of water and 140 cc. of ethanol; some insoluble material was separated by filtration. Upon cooling, crystals deposited which were washed bromine-free with ethanol. The yield was 2.1 g. (74%) of prismatic needles which, after recrystallization from aqueous 80% ethanol, melted in an evacuated capillary tube at 332–334° (dec.).

Anal. Calcd. for $C_9H_{13}O_5N_2SNa$: C, 38.02; H, 4.61; Na, 8.09. Found: C, 38.17; H, 4.45; Na, 8.29.

Sodium Salt of 4-Methyl-5-(ϵ-sulfoamyl)-2-imidazolidone (I, R = SO_3Na).—The foregoing imidazolone (1.68 g.) was hydrogenated at room temperature and atmospheric pressure with 1 g. of prehydrogenated Adams platinum catalyst in 15 cc. of acetic acid. Two moles of hydrogen were taken up in one hour, a third mole overnight, whereby the material, initially in suspension, passed into solution. After separation of the catalyst, the solution was evaporated to a sirup, which was rendered free of acetic acid by evaporation with methanol. The final residue was dissolved in 25 cc. of hot methanol. Addition of 25 cc. of dry ether and cooling produced 1.09 g. of crystals (68%). The substance was recrystallized for analysis by dissolving in methanol and adding ether. It did not show a distinct melting point.

Anal. Calcd. for $C_9H_{17}O_4N_2SNa$: C, 39.64; H, 6.29;

(1) Roblin, *Chem. Rev.*, **38**, 377 (1946).

(2) We had accomplished the present work when Hofmann, Bridgwater and Axelrod, THIS JOURNAL, **69**, 1550 (1947), reported the synthesis of a sulfonic acid analog of oxybiotin.

(3) Duschinsky and Dolan, *ibid.*, **67**, 2079 (1945).

(4) Rubin, Drekter and Moyer, *Proc. Soc. Exp. Biol. Med.*, **58**, 352 (1945); Rubin, Flower, Rosen and Drekter, *Arch. Biochem.*, **8**, 79 (1945).

(5) Axelrod, De Woody and Hofmann, *J. Biol. Chem.*, **163**, 771 (1946); Hofmann, Chen, Bridgwater and Axelrod, THIS JOURNAL, **69**, 191 (1947).

(6) Melting points were determined with an uncalibrated set of Anschütz thermometers.

(7) Hunsdiecker and Hunsdiecker, *Ber.*, **75**, 296 (1942).

(8) Merchant, Wickert and Marvel, THIS JOURNAL, **49**, 1830 (1927).

S, 11.78; Na, 8.45. Found: C, 39.06, 39.30; H, 6.06, 5.74; S, 11.38; Na, 8.22.

Acknowledgment.—The authors are indebted to Dr. Al Steyermark for the microanalyses and to Mr. Jacob Scheiner for the microbiological assays.

Summary

A sulfonic acid analog of desthiobiotin was synthesized. The compound showed inhibitory activity toward *S. cerevisiae*, which was more pronounced against *d,l*-O-heterobiotin and *d,l*-desthiobiotin than *d*-biotin. The compound had no effect on *L. casei*.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

The Reaction of Some Radioactive Mustard-type Vesicants with Purified Proteins[1]

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In a collaborative attack on the problem of the mechanism of action of mustard-type vesicants, we had undertaken to study the reaction of vesicants with proteins. A study of the reactions of a large number of amino acids with vesicants has already been described.[4] The second phase of our work was the study of the interaction of vesicants with certain well characterized, highly purified proteins. Preliminary experiments indicated that treatment of certain proteins with relatively large amounts of vesicant resulted in a chemical reaction. The reaction products differed from the original proteins in physical properties and had a higher sulfur content. These observations supplemented earlier evidence[5] of chemical reactions between vesicants and proteins and indicated the desirability of a detailed study.

Kistiakowsky, Moritz, Henriques and co-workers[6] had already demonstrated that an extremely small amount of mustard gas (H) is bound in the tissue at the site of a burn produced by a minimum amount of H. At the same time, all indications were that the vesicants were capable of reacting with a large number of different types of groups presumably present in proteins. Reactions at the site of the burn must involve only a small percentage of these groups and possibly only certain types. It was, therefore, of particular interest to study the reaction of H-type vesicants with proteins *in vitro* at correspondingly low ratios of vesicant to protein in an effort to determine the most reactive groups under these conditions. It was decided to utilize the radioactive tracer technique in approaching this question.

(1) The work described in this paper was carried out under Contract OEMsr-144 between the Office of Scientific Research and Development and Cornell University Medical College, and is described in Progress Reports to the National Defense Research Committee, January, 1942, to November, 1943.

(2) Present address: School of Biological Sciences, The University of Tennessee, Memphis, Tenn.

(3) Present address: Department of Chemistry, State College of Washington, Pullman, Wash.

(4) du Vigneaud, Stevens, McDuffie, Wood and McKennis, THIS JOURNAL, **70**, 1620 (1948).

(5) (a) Berenblum and Wormall, *Biochem. J.*, **33**, 75 (1939); (b) unpublished British Reports: Berenblum (1940), Pirie (1941), Peters (1941).

(6) Progress Reports to NDRC Section B4C (1942).

Benzyl β-chloroethyl sulfide (benzyl-H*) and *n*-butyl β-chloroethyl sulfide (butyl-H*), containing S^{35} of 87-day half-life,[7] were synthesized from benzyl mercaptan* and butyl mercaptan*, respectively. The general scheme for the synthesis of the vesicants is shown

$$RMgX \xrightarrow{S^*} RS^*MgX \xrightarrow[H^+]{HOH} RS^*H \xrightarrow[ClCH_2CH_2OH]{NaOH}$$

$$RS^*CH_2CH_2OH \xrightarrow{HCl} RS^*CH_2CH_2Cl$$

A number of syntheses of these compounds containing isotopic sulfur have already been described.[8] The procedure employed by us in this investigation contains technical features which facilitated the handling of the small amounts of materials involved, and is, therefore, presented in some of its details.

The estimations of radioactivity were carried out essentially by the method of Henriques and coworkers.[9] The radioactivity of the sulfur in the vesicants used to treat the proteins was sufficient to allow the detection of as little as 5×10^{-6} mg. of benzyl-H* or butyl-H* residues per milligram of protein sulfur in the protein derivative or its hydrolysis products.

The proteins utilized were crystalline insulin, crystalline pepsin and crystalline tobacco mosaic virus.

Insulin.—Insulin was selected for study particularly because of its unique physiological activity, and because a large amount of chemical data on the molecule is available. Furthermore, it has no known organic constituents other than amino acids.

Insulin was treated with benzyl-H* or butyl-H* in amounts ranging from 0.25 to 4.0 mg. of vesicant per 100 mg. of protein. This resulted in insulin-vesicant* preparations containing from 0.3 to

(7) An asterisk (*) is used to indicate the presence of radiosulfur in a compound.

(8) Tarver and Schmidt, *J. Biol. Chem.*, **146**, 69 (1942); Seligman, Rutenburg and Banks, *J. Clin. Investigation*, **22**, 275 (1943); Kilmer and du Vigneaud, *J. Biol. Chem.*, **154**, 247 (1944).

(9) Henriques, Kistiakowsky, Margnetti and Schneider, *Ind. Eng. Chem., Anal. Ed.*, **18**, 349 (1946).