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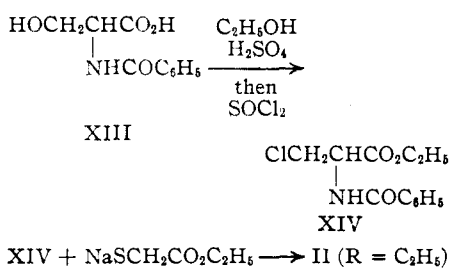
Biotin. II. Synthesis of Biotin

BY STANTON A. HARRIS, DONALD E. WOLF, RALPH MOZINGO, R. CHRISTIAN ANDERSON, GLEN E. ARTH, NELSON R. EASTON,[1] DOROTHEA HEYL, ANDREW N. WILSON AND KARL FOLKERS

Comparison of synthetic biotin[2] with natural biotin verified earlier conclusions[3,4,5] that biotin has structure XII.

The reactions used for synthesis of biotin are summarized below. The details of this synthesis, the relationship of the various isomeric forms and the side reactions encountered are described in accompanying[6] papers and other papers to follow.

The starting materials for this synthesis are *l*-cystine, chloroacetic acid and glutaric acid. The *l*-cystine was reduced in liquid ammonia with sodium and then coupled with chloroacetic acid to give β-(carboxymethylmercapto)-alanine, I.[7] Subsequent benzoylation and esterification yielded the dimethyl ester of N-benzoyl-β-(carboxymethylmercapto)-alanine, II. The diethyl ester was obtained by condensing thioglycolic ester with the ethyl ester of N-benzoyl-β-chloroalanine, XIV, derived from N-benzoylserine,[8] XIII, by esterification and chlorination with thionyl chloride.



The dimethyl ester, II, was treated with sodium methoxide in methanol to give the sodium salt of 4-benzamido-3-ketotetrahydro-2-thiophenecarboxylic acid methyl ester, III. Racemization occurred during this reaction. The sodium salt was hydrolyzed and decarboxylated in an aqueous acetic acid-

(1) Present address: Department of Chemistry, University of Illinois, Urbana, Illinois.

(2) Harris, Wolf, Mozingo and Folkers, *Science*, **97**, 447 (1943). This is the first paper in this series.

(3) Refer to review papers by du Vigneaud (*ibid.*, **96**, 455 (1942)) and Hofmann ("Advances in Enzymology," Vol. 3, p. 289 (1943), Interscience Publishers, Inc., New York).

(4) du Vigneaud, Melville, Folkers, Wolf, Mozingo, Keresztesy and Harris, *J. Biol. Chem.*, **146**, 475 (1942).

(5) Melville, Moyer, Hofmann and du Vigneaud, *ibid.*, **146**, 487 (1942).

(6) (a) Harris, Mozingo, Wolf, Wilson, Arth and Folkers, THIS JOURNAL, **66**, 1800 (1944). (b) Harris, Easton, Heyl, Wilson and Folkers, *ibid.*, **66**, 1757 (1944).

(7) (a) Michaelis and Shubert, *J. Biol. Chem.*, **106**, 331 (1934); (b) Blood and Lewis, *ibid.*, **139**, 407 (1941).

(8) Sorenson and Anderson, *Z. physiol. Chem.*, **56**, 297 (1908).

hydrochloric acid solution to give 4-benzamido-3-ketotetrahydrothiophene, IV.

The valeric acid side chain was introduced by means of an aldehyde prepared from glutaric acid.[9] The acid was converted in turn to glutaric anhydride, glutaric acid monomethyl ester, γ-carbomethoxybutyryl chloride,[10] V, and finally to methyl γ-formylbutyrate by a Rosenmund reduction.[11]

The aldehyde ester, VI, condensed with the ketone, IV, when piperidine acetate was used as the catalyst, to yield the methyl ester of 4-benzamido - 3 - keto - $\Delta^{2,5}$ - tetrahydro - 2 - thiophenevaleric acid, VII, m. p. 116° (*Anal.* Calcd. for $C_{17}H_{19}NO_4S$: C, 61.24; H, 5.74; N, 4.20. Found: C, 61.37; H, 6.05; N, 4.17). This unsaturated ketone, VII, reacted with hydroxylamine hydrochloride in pyridine to yield the methyl ester of 4-benzamido-3-oximino-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid, VIII. The unsaturated oxime, VIII, was reduced in an acetic acid-acetic anhydride mixture with zinc dust. Two dehydro compounds were obtained, m. p. 185–186° and m. p. 162–163°. One of these, m. p. 185–186°, is the methyl ester of 3-acetamido-4-benzamido-4,5-dihydro-2-thiophenevaleric acid, IX (*Anal.* Calcd. for $C_{19}H_{24}N_2O_4S$: C, 60.61; H, 6.42; N, 7.43; S, 8.52. Found: C, 60.79; H, 6.33; N, 7.45; S, 8.86). The position of the double bond in this compound will be discussed in a later paper.

This dehydro compound, IX, was hydrogenated over a palladium catalyst. By fractional crystallization of the products, two racemates, m. p. 153–154° (*Anal.* Calcd. for $C_{19}H_{26}N_2O_4S$: C, 60.29; H, 6.92; N, 7.43. Found: C, 60.40; H, 6.92; N, 7.32) and m. p. 172–173°, of the methyl ester of 3-acetamido-4-benzamidotetrahydro-2-thiophenevaleric acid, X, were obtained. After hydrolysis of each of these diamido esters, X, with barium hydroxide, as was done with biotin,[12] and subsequent treatment with sulfuric acid, the corresponding sulfates of the 3,4-diaminotetrahydro-2-thiophenevaleric acids were obtained. Treatment of these diamino acids, XI, with phosgene[13] yielded two racemates of hexahydro - 2 - oxo - 1 - thieno[3, 4]imidazole - 4 - valeric acid, XII, which will be called *dl*-biotin, m. p. 232°, and *dl*-allobiotin, m. p. 194–196°. *dl*-Biotin was derived from the diamido ester, X, melting at 153–154°. *dl*-Biotin was resolved through its esters with *l*-mandelic acid to give biotin.[2]

(9) "Organic Syntheses," Coll. Vol. I, 2nd ed., p. 289 (1941).
(10) Clutterbuck and Raper, *Biochem. J.*, **19**, 385 (1925).
(11) "Organic Syntheses," **21**, 84 (1941).
(12) Hofmann, Melville and du Vigneaud, *J. Biol. Chem.*, **141**, 207 (1941).
(13) Melville, Hofmann and du Vigneaud, *Science*, **94**, 308 (1941).

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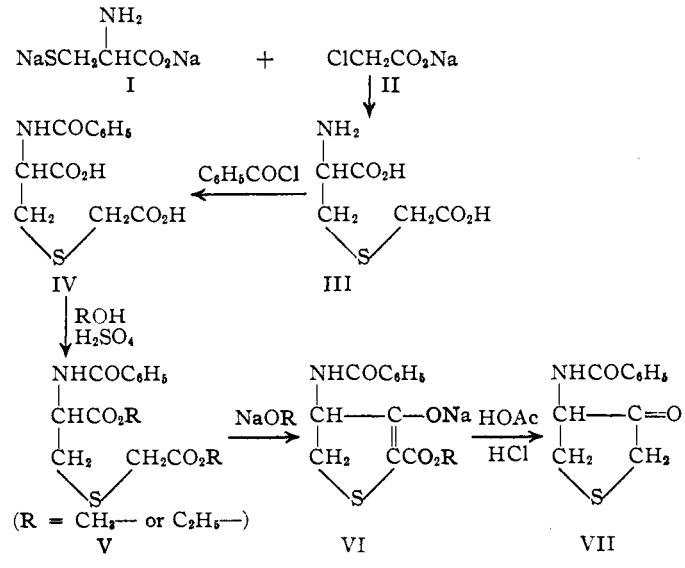
Biotin. IV. Synthesis of 4-Benzamido-3-ketotetrahydrothiophene

BY STANTON A. HARRIS, NELSON R. EASTON, DOROTHEA HEYL, ANDREW N. WILSON AND KARL FOLKERS

A summary of the reactions used in the total synthesis of biotin was given in a previous communication.[1] The synthesis of the 4-benzamido - 3 - ketotetrahydrothiophene, which is a key intermediate in this synthesis, was obtained by the reactions described in this paper.

l-Cystine, or *l*-cysteine, and chloroacetic acid are the primary starting materials for this synthesis. *l*-Cysteine and chloroacetic acid were condensed previously[2] in alkaline solution to give β-(carboxymethylmercapto)-alanine, III. The benzoylation and esterification to compounds IV and V were accomplished without racemization. The ring closure of the ester, V, was a very facile reaction, since it took place in methanol at room temperature by adding sodium methoxide. After a few seconds, the crystallization of the sodium salt, VI, commenced and the reaction was



(1) Harris, Wolf, Mozingo, Anderson, Arth, Easton, Heyl, Wilson and Folkers, THIS JOURNAL, **66**, 1756 (1944).
(2) (a) Michaelis and Shubert, *J. Biol. Chem.*, **106**, 331 (1934); (b) Blood and Lewis, *ibid.*, **139**, 407 (1941).