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Biotin. V. Synthesis of *dl*-Biotin, *dl*-Allobiotin and *dl-epi*-Allobiotin

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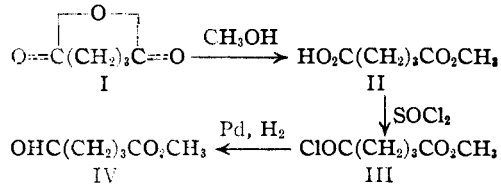
dl-Biotin and two stereoisomeric racemates, *dl*-allobiotin and *dl-epi*-allobiotin, have been synthesized[2] from 4-benzamido-3-ketotetrahydrothiophene[3] and methyl γ-formylbutyrate, IV, by the reactions described in this paper.

The methyl γ-formylbutyrate, IV, required for the introduction of the valeric acid side chain, was prepared from glutaric anhydride which was converted in turn to methyl hydrogen glutarate, II, γ-carbomethoxybutyryl chloride, III, and finally methyl γ-formylbutyrate, IV. This aldehyde was characterized as the 2,4-dinitrophenylhydrazone.



Once the methyl γ-formylbutyrate had been obtained halogen free, it showed no signs of polymerization and gave consistent results in condensation with 4-benzamido-3-ketotetrahydrothiophene, V, to yield 4-benzamido-3-keto-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid methyl ester, VI.

The unsaturated keto ester, VI, reacted with hydroxylamine in pyridine to yield the methyl ester of 4-benzamido-3-oximino-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid, VII. This product was separated by fractional crystallization into two isomeric oximes. One of these oximes was needle-like in form and melted at 159–160° while the other crystallized in small plates which melted at 145–146°. They may be *syn* and *anti* forms of the oxime. However, it was unnecessary to separate the oximes since the mixture gave the same products in about the same yield as each of the pure isomers in the succeeding step.

The mixed unsaturated oximes, VII, were reduced in an acetic acid–acetic anhydride mixture with zinc dust to yield two isomeric products. One of these, m. p. 185–186°, appears to be the methyl ester of 3-acetamido-4-benzamido-4,5-dihydro-2-thiophenevaleric acid, VIII, and will be referred to hereafter as the *dl*-isodehydro ester. The second one, m. p. 162–163°, seems to be the methyl ester of 3-acetamido-4-benzamido-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid, IX, and will be called the *dl*-allodehydro ester. Evidence for the position of the double bond in these dehydro isomers was obtained from their behavior upon hydrolysis and by the determination of the spatial arrangement of the nitrogen atoms in the fully hydrogenated products. The detailed discussion of the structure of these dehydro isomers is presented in connection with the correlation of the isomers[4] obtained in the biotin synthesis.

Each of the dehydro esters, VIII and IX, was hydrogenated over a palladium catalyst.[5] By fractional crystallization of the products, two racemates of the methyl ester of 3-acetamido-4-benzamido-tetrahydro-2-thiophenevaleric acid, X, which will be referred to as diamido esters, were obtained in each case. The *dl*-isodehydro ester, m. p. 185–186°, yielded the *dl*-diamido ester, m. p. 152–153°, and the *dl*-allodiamido ester, m. p. 172–173°. The *dl*-diamido ester is the precursor of *dl*-biotin. The *dl*-allodehydro ester, m. p. 162–163°, yielded both the *dl*-allodiamido ester, m. p. 172–173°, and the *dl-epi*-allodiamido ester, m. p. 185–187°. Table I summarizes all of the products in the three series of compounds derived from the two dehydro esters.

TABLE I

NOMENCLATURE AND MELTING POINTS OF THE ISOMERIC COMPOUNDS IN THE BIOTIN SYNTHESIS

Dehydro ester	*dl*-Iso, VIII 185–186° → *dl*-, °C.	*dl*-Allo, °C.	*dl*-Allo, IX 162–163° → *dl-epi*-Allo-, °C.
Diamido ester, X	155	172–173	185–187
Diamido acid, XI	232	195[a]	189–190[a]
Sulfate of diamino acid, XII	249–250	228–230	283–285
Biotin, XIII	232	194–196[b]	Fuses above 195[b]

[a] The mixed melting point of these two compounds was 182–184°. [b] The mixed melting point of these two compounds was depressed to about 180°.

The three racemic diamido esters, X, were hydrolyzed in an aqueous alcohol solution of sodium hydroxide to give the corresponding racemic diamido acids, XI. When the racemic diamido esters or acids were hydrolyzed in barium hydroxide solution at 140°, as was done with biotin,[6] the corresponding racemic 3,4-diaminotetrahydro-2-thiophenevaleric acids, XII (*dl*-diamino acid, *dl*-allodiamino acid and *dl-epi*-allodiamino

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

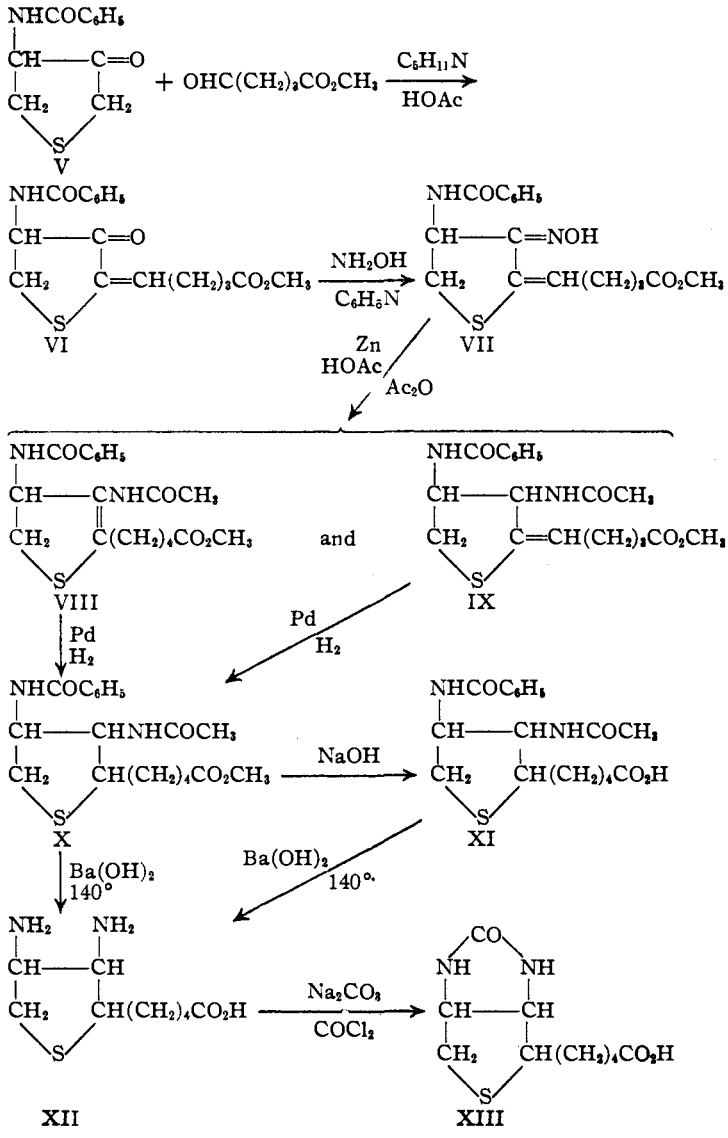
(2) Harris, Wolf, Mozingo, Anderson, Arth, Easton, Heyl, Wilson and Folkers, THIS JOURNAL, **66**, 1756 (1944).

(3) Harris, Easton, Heyl, Wilson and Folkers, *ibid.*, **66**, 1757 (1944).

(4) Harris, Mozingo, Wolf, Wilson and Folkers, *ibid.*, **67**, 2102 (1945).

(5) Mozingo, Harris, Wolf, Hoffhine, Easton and Folkers, *ibid.*, **67**, 2092 (1945).

(6) Hofmann, Melville and du Vigneaud, *J. Biol. Chem.*, **141**, 207 (1941).



acid) were obtained. These compounds were isolated as their sulfates. Treatment of these three racemic diamino acids, XII, with sodium carbonate and phosgene as was done in the resynthesis of biotin[7] yielded three racemates (*dl*-biotin, *dl*-allobiotin and *dl-epi*-allobiotin) stereochemically related to biotin.

The isolation of these racemates made it necessary to adopt a system of nomenclature for them. The name biotin was originally suggested by Kögl and Tönnis[8] for the "native active material." A satisfactory basis for naming the isomers is to designate the enantiomorph of biotin as *l*-biotin (where *l* denotes the sign of rotation) and the corresponding racemate, *dl*-biotin.[9] The name biotin when used for the biologically active isomer does not necessarily require the prefix *d* since the optical property is included in the original definition. The second racemate is designated *dl*-allobiotin and the third racemate is called *dl-epi*-allobiotin. The precursors of each of these compounds have been given the corresponding prefix. The nomenclature of all of the products obtained in isomeric forms in the synthesis of *dl*-biotin is summarized in Table I.

dl-Allobiotin and *dl-epi*-allobiotin are configurationally identical to each other about the asymmetric carbon atoms to which the nitrogen atoms are attached.[10]

During the synthesis of *dl*-biotin it was necessary to determine which of the various fractions of the diamido esters contained the precursor of biotin. A micro method for this purpose which was reasonably rapid was adapted from the barium hydroxide hydrolysis[6] and phosgene reactions.[7] The substitution of sodium hydroxide for the barium hydroxide offered no advantage in a Pyrex tube because of the formation of silicic acid. A sample of 10 mg. of a given product was hydrolyzed and treated with phosgene without the isolation of any product and assayed for its growth promoting power toward *Lactobacillus arabinosus* No. 17-5. The corresponding reactions were performed upon the dibenzoyl derivative of the diaminocarboxylic acid[6] as a control, and an estimate of the biotin content of the samples was made by comparison with a standard solution of biotin.[11]

dl-Biotin showed 50% of the activity of biotin, while *dl*-allobiotin and *dl-epi*-allobiotin were essentially inactive under comparable conditions in the *L. arabinosus* assay.[12] The *dl*-diamino acid showed 1.8% of the activity of biotin. In bioassays with rats[13] in which biotin deficiency had been induced by the feeding of raw egg white and in the prophylactic assay with chicks,[14] *dl*-biotin showed 50% of the activity of biotin, while *dl*-allobiotin and the diamino acids showed no activity.

(7) Melville, Hofmann and du Vigneaud, *Science*, **94**, 308 (1941).

(8) Kögl and Tönnis, *Z. physiol. Chem.*, **242**, 43 (1936).

(9) By private communication, Professor Vincent du Vigneaud was in agreement with this nomenclature. Unfortunately, it was impossible to communicate with Professor F. Kögl at the time of this decision.

(10) Harris, Mozingo, Wolf, Anderson, Wilson, Arth and Folkers, THIS JOURNAL, **66**, 1800 (1944).

(11) These assays, as well as those on the purified isomers, were carried out by Dr. Jacob L. Stokes and Misses Marion Gunness and Muriel Caswell of this Laboratory.

(12) Stokes and Gunness, *J. Biol. Chem.*, **157**, 121 (1945).

(13) Emerson, *ibid.*, **157**, 127 (1945).

(14) Ott, *ibid.*, **157**, 131 (1945).

Experimental

Methyl Hydrogen Glutarate.[15]—A mixture of 412 g. of glutaric anhydride and 237 g. of methyl alcohol was heated for two hours on a steam-bath. The reaction is quite exothermic. The product was distilled from a Claisen flask under reduced pressure. A small fore-run came over followed by the half-ester, b. p. 158–165° at 23 mm. The yield was 487 g. (92%).

γ-Carbomethoxybutyryl Chloride.[15]—Four hundred grams of pure thionyl chloride was added to 487 g. of methyl hydrogen glutarate, and the mixture allowed to stand until evolution of gas was slow after which it was heated on the steam-bath to complete the reaction. The residue was fractionated through a Widmer column under reduced pressure, the portion boiling at 110–113° at 23 mm. being collected. The yield was 522 g. (87%).

Methyl γ-Formylbutyrate.—The reduction of γ-carbomethoxybutyryl chloride was carried out by a method described for the preparation of 2-naphthaldehyde[16] using 330 g. of this chloride, 50 g. of 5% palladium–barium sulfate and 5 cc. of the quinoline–sulfur poison[16] in 1200 cc. of pure xylene. The reduction required about four hours at reflux temperature. After centrifuging the catalyst and removing the solvent under reduced pressure, the residue was fractionated. The yield of aldehyde boiling at 100–103° at 23 mm. corresponded to 70–85% of a product containing some hydrogen chloride and combined halogen.

This aldehyde was not suitable for use in the condensation reaction without further purification because of its tendency to polymerize; the polymer was partially depolymerized by distilling from a trace of *p*-toluenesulfonic acid. The halogenated products were removed by dissolving the aldehyde in benzene and adding pyridine until no more precipitation occurred. The solution was washed with water, was dried with sodium sulfate and was distilled under reduced pressure. In one run 221 g. of crude aldehyde was obtained. After purification, the yield of aldehyde was 136 g. (52%).

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.32; H, 8.02.

2,4-Dinitrophenylhydrazone of Methyl γ-Formylbutyrate.—A sample of the xylene solution of the crude aldehyde was treated with 2,4-dinitrophenylhydrazine. After two days, crystals of the hydrazone separated and were filtered, washed with ethanol and dried; m. p. 105–106°.

Anal. Calcd. for $C_{12}H_{14}N_4O_6$: C, 46.46; H, 4.55; N, 18.06. Found: C, 47.00, 46.72; H, 4.37, 4.29; N, 18.10.

Methyl Ester of 4-Benzamido-3-keto-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric Acid, VI.—A suspension of 133 g. of 4-benzamido-3-ketotetrahydrothiophene, V, in 600 cc. of methyl alcohol was allowed to react with 78 g. of methyl γ-formylbutyrate, IV, using 10 cc. of piperidine and 5 cc. of acetic acid as the catalyst. The temperature of the reaction mixture rose to about 40° and the ketone went into solution after about one-half hour. Soon afterward, the unsaturated keto ester, VI, started to crystallize. After standing for three to four hours between 30 and 40°, the mixture was cooled and kept in the refrigerator overnight. The methyl ester of 4-benzamido-3-keto-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid, VI, was collected, was washed with ethyl ether and was dried; m. p. 116°; yield 106 g. (53%).

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.

Methyl Ester of 4-Benzamido-3-oximino-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric Acid, VII.—A mixture of 285 g. of the unsaturated keto ester, VI, and 90 g. of finely ground hydroxylamine hydrochloride was dissolved in 600 cc. of pyridine. After fifteen hours at 30°, the reaction mixture was diluted with four times its volume of water and extracted five times with 100-cc. portions of chloroform. Cracked ice was added to the chloroform solution in a separatory funnel and the mixture was shaken with 2.5 *N* hydrochloric acid until the aqueous layer was just acid to congo red. After washing twice with water, the chloroform solution was dried over anhydrous sodium sulfate, was filtered and was concentrated to a small volume at 40°. When the last traces of chloroform were removed with a Hyvac pump, the light-brown mass partially crystallized to a sticky semi-solid mass. Ether (250 cc.) was added and the lumps were crushed with a spatula. Nearly all of the colored matter dissolved in the ether. The white, powdered mixed oximes were filtered, washed twice with ether and dried; m. p. 129–138°; yield, 176 g. (59%).

By repeated crystallization of the mixed oximes from methyl alcohol, a voluminous needle-like crystalline oxime, m. p. 159–160°, was obtained.

Anal. Calcd. for $C_{17}H_{20}N_2O_4S$: C, 58.60; H, 5.79; N, 8.04. Found: C, 58.77; H, 5.93; N, 8.11.

The methyl alcohol mother liquors were evaporated to dryness and repeated crystallizations of the residue from isopropyl alcohol produced a second oxime, m. p. 145–146°, which crystallized in small plates. A mixture of these two oximes melted at 138–144°.

Anal. Calcd. for $C_{17}H_{20}N_2O_4S$: C, 58.60; H, 5.79; N, 8.04. Found: C, 58.77; H, 5.68; N, 8.14.

These products were probably *syn* and *anti* isomers of the methyl ester of 4-benzamido-3-oximino-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid, VII. It was found that the mixed oximes could be used in the succeeding steps without separation.

Methyl Ester of Racemic 3-Acetamido-4-benzamido-4,5-dihydro-2-thiophenevaleric Acid, VIII.—A mixture of 20.5 g. of mixed oximes, VII, in 125 cc. of acetic acid and 125 cc. of acetic anhydride was stirred with 24 g. of zinc dust in an ice-bath for seven and one-half hours. The zinc and zinc acetate were removed by filtration and the acetic acid and acetic anhydride were distilled at water-pump pressure. When the residue started to crystallize, 100 cc. of ether was added and the lumps were crushed. After shaking vigorously, the ether solution was filtered and the residue, melting at 179–183°, was recrystallized from methyl alcohol to yield 7.48 g. of product melting at 184–185°. After recrystallization from methyl alcohol, the melting point was constant at 185–186°. This compound appears to be the methyl ester of 3-acetamido-4-benzamido-4,5-dihydro-2-thiophenevaleric acid, VIII (*dl*-isodehydro ester).

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, 60.43, 60.50; H, 6.33, 6.23, 6.26; N, 7.45; S, 8.86.

Methyl Ester of Racemic 3-Acetamido-4-benzamido-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric Acid, IX,—The ether mother liquor from the preceding experiment deposited 5.2 g. of crystals, m. p. 150–160°, on standing in the refrigerator. Recrystallization from methyl alcohol raised the melting point to 162–163°. This compound probably is the methyl ester of 3-acetamido-4-benzamido-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid, IX (*dl*-allodehydro ester).

Anal. Calcd. for $C_{19}H_{24}N_2O_4S$: C, 60.61; H, 6.42; N, 7.43. Found: C, 60.46; H, 6.43; N, 7.65.

Methyl Esters of Racemic 3-Acetamido-4-benzamido-tetrahydro-2-thiophenevaleric Acids, X

(a) ***dl*-Allodiamido Ester.**—One and one-tenth grams of the *dl*-isodehydro ester, m. p. 185–186°, was dissolved in 125 cc. of methyl alcohol which contained 7 g. of palladium on charcoal catalyst.[5] The calculated amount of hydrogen was absorbed after shaking for twenty minutes at 40 lb. pressure. The catalyst was removed by filtration and was thrice extracted with boiling methyl alcohol. After concentration, the dry weight of this fraction was 0.4 g. Recrystallization of this fraction from methyl alcohol yielded pure *dl*-allodiamido ester, X, m. p. 172–173°.

Anal. Calcd. for $C_{19}H_{26}N_2O_4S$: C, 60.29; H, 6.92; N, 7.43. Found: C, 60.27; H, 6.83; N, 7.23.

(b) ***dl*-Diamido Ester.**—The catalyst from the above experiment was twice extracted with chloroform to yield

(15) Clutterbuck and Raper, *Biochem. J.*, **19**, 385 (1925).

(16) "Organic Syntheses," **21**, 84 (1941).

0.4 g. of an oil which was crystallized from methyl alcohol by adding water. It was recrystallized by dissolving in a large volume of ether and concentrating until crystallization commenced. After three such crystallizations, the melting point of the *dl*-diamido ester was 152–153°.

Anal. Calcd. for $C_{16}H_{26}N_2O_4S$: C, 60.29; H, 6.92; N, 7.43. Found: C, 60.40; H, 6.92; N, 7.32.

A suspension of 40 g. of the *dl*-isodehydro ester and 185 g. of 5% palladium–barium sulfate catalyst[6] in 3500 cc. of methyl alcohol was shaken for forty-two hours at 30° under 40 lb. hydrogen pressure. Since reduction was incomplete, the catalyst was removed by filtration and a fresh lot of 185 g. of the catalyst was added. After an additional twenty-four hours the reduction was complete. The catalyst was removed by centrifuging and was washed with methyl alcohol. The solution was concentrated to 150 cc. and allowed to stand in a refrigerator overnight. A yield of 7.7 g. of the *dl*-allodiamido ester, m. p. 168–170°, was obtained. After further concentration a crop of material melting at 135–140° was obtained; yield, 28 g. (68%). Although this material was not pure *dl*-diamido ester, it gave *dl*-biotin in 60% yield.

(c) ***dl-epi*-Allodiamido Ester.**—A solution of 0.55 g. of the *dl*-allodehydro ester, m. p. 162–163°, was reduced with palladium on Darco in methyl alcohol as described for the *dl*-isodehydro ester. The reduction was complete in fifty minutes. Less than 200 mg. of material was present in the solvent. The remainder was eluted from the charcoal catalyst with several portions of boiling ethyl alcohol and finally with two portions of chloroform. The eluate was evaporated to dryness and the residue was recrystallized from ethyl alcohol to give the *dl-epi*-allodiamido ester in elongated rods with square ends; m. p. 185–187°.

Anal. Calcd. for $C_{16}H_{26}N_2O_4S$: C, 60.29; H, 6.92; N, 7.43; S, 8.47. Found: C, 60.27; H, 7.24; N, 7.44; S, 8.36.

Fractional crystallization of the products in the mother liquors yielded *dl*-allodiamido ester, melting at 172–173°, which did not depress the melting point of the *dl*-allodiamido ester obtained above.

The Racemic 3-Acetamido-4-benzamidotetrahydro-2-thiophenevaleric Acids, XI

(a) ***dl*-Allodiamido Acid.**—The *dl*-allodiamido ester, m. p. 172–173°, was hydrolyzed in a 50% methyl alcohol–water solution with one equivalent of sodium hydroxide. After heating for one-half hour on a steam-bath, the alcohol was removed by distillation and the solution was acidified with hydrochloric acid, whereupon the acid crystallized. The *dl*-allodiamido acid was recrystallized from acetone; m. p. 195°.

Anal. Calcd. for $C_{16}H_{24}N_2O_4S$: C, 59.32; H, 6.64; N, 7.69. Found: C, 59.38; H, 6.78; N, 7.57.

(b) ***dl*-Diamido Acid.**—The *dl*-diamido ester, m. p. 152–153° was hydrolyzed and recrystallized as described above. The *dl*-diamido acid melted at 232°.

Anal. Calcd. for $C_{16}H_{24}N_2O_4S$: C, 59.32; H, 6.64; N, 7.69. Found: C, 59.30, 59.16; H, 6.85, 6.53; N, 7.81.

(c) ***dl-epi*-Allodiamido Acid.**—The *dl-epi*-allodiamido ester, m. p. 183–185°, yielded the *dl-epi*-allodiamido acid which was recrystallized from hot water; m. p. 192°.

Anal. Calcd. for $C_{16}H_{24}N_2O_4S$: C, 59.32; H, 6.64; N, 7.69. Found: C, 59.46, 59.59; H, 7.14, 6.96; N, 7.62.

Sulfates of the Racemic 3,4-Diaminotetrahydro-2-thiophenevaleric Acids, XII

(a) **Sulfate of *dl*-Allodiamino Acid.**—A mixture of 280 mg. of the *dl*-allodiamido ester, m. p. 172–173°, and 28 cc. of water was heated with 2.8 g. of barium hydroxide at 140–150° for fifteen hours. After removal of the barium ion with sulfuric acid, the benzoic acid was extracted with ether and the solution evaporated nearly to dryness. The sulfate of *dl*-allodiamino acid crystallized and was recrystallized from water–methyl alcohol in microcrystalline needles; m. p. 228–230°.

Anal. Calcd. for $C_8H_{20}N_2O_6S_2$: C, 34.16; H, 6.37; N, 8.85. Found: C, 34.09; H, 6.42; N, 8.93.

(b) **Sulfate of *dl*-Diamino Acid.**—The *dl*-diamido ester, m. p. 152–153°, yielded the sulfate of *dl*-diamino acid which crystallized in plates from water–methyl alcohol; m. p. 249–250°.

Anal. Calcd. for $C_8H_{20}N_2O_6S_2$: C, 34.16; H, 6.37; N, 8.85. Found: C, 34.44; H, 6.66; N, 8.86.

(c) **Sulfate of *dl-epi*-Allodiamino Acid.**—The *dl-epi*-allodiamido ester, m. p. 185–187°, yielded the sulfate of *dl-epi*-allodiamino acid, which melted at 283–285° after recrystallization from water.

Anal. Calcd. for $C_8H_{20}N_2O_6S_2$: C, 34.16; H, 6.37; N, 8.85. Found: C, 34.22; H, 6.32; N, 8.85.

The Racemic Hexahydro-2-oxo-1-thieno[3,4]-imidazole-4-valeric Acids, XII

(a) ***dl*-Allobiotin.**—Forty-five milligrams of the *dl*-allodiamino acid sulfate, m. p. 228–230°, was converted to the ureide as described previously for biotin[7]; yield, 25 mg.; m. p. 194–196°.

Anal. Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.36; H, 6.50; N, 11.39.

The yield of *dl*-allobiotin from 3 g. of the *dl*-allodiamido acid, m. p. 195°, without the isolation of the intermediate diamine was 1.1 g. (54%) after one recrystallization from water.

(b) ***dl*-Biotin.**—The *dl*-diamido acid, m. p. 232°, (0.5 g.) was placed in a sealed tube with 5 g. of hydrated barium hydroxide and 25 cc. of water and heated at 140° for sixteen hours. The resulting mixture was acidified to congo red with sulfuric acid and centrifuged to remove barium sulfate. The filtrate and washings from the barium sulfate were concentrated to about 100 cc. and treated with 5 g. of sodium carbonate. This solution was treated with phosgene at 0° until the solution was acid to congo red. The racemic biotin crystallized and was filtered and dried; yield 0.29 g. (89%); m. p. 232° after recrystallization from water. This material showed slightly greater than 50% of the activity of natural biotin.

Anal. Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.33; H, 6.39; N, 11.68.

In another experiment, 6 g. of crude diamido ester, m. p. 135–140°, was hydrolyzed with 60 g. of barium hydroxide octahydrate in 300 cc. of water at 140° for fourteen hours. The mixture was acidified to congo red with sulfuric acid and the barium sulfate was removed by filtration through filtercel. This filtrate was concentrated to 200 cc., shaken with ether to remove benzoic acid and filtered with about 5 g. of Darco G-60 to remove the yellow color. The solution was neutralized with sodium carbonate and then 20 g. of sodium carbonate was added and the solution was filtered to remove a cloudy precipitate. The solution was kept below 10° and phosgene was bubbled in with stirring until the mixture became permanently acid to congo red. The precipitated *dl*-biotin, m. p. 220–232°, was removed; yield 2.4 g. (60%). An additional amount of *dl*-biotin was obtained by treating the mother liquors with 10 g. of Darco G-60. The charcoal was removed by filtration, washed well with water and eluted with 100 cc. of about 5 *N* ammonium hydroxide. After evaporation of the eluate, 0.6 g. of material was recovered making a total yield of 77% of crude material.

The *dl*-biotin obtained directly from the phosgene treatment of the *dl*-diamino acid from this crude *dl*-diamido ester contained a little gummy material and was recrystallized. Some of this material (1.1 g.) melting at 220–232° was recrystallized from 200 cc. of boiling water. By cooling the solution a first crop of 0.6 g. of *dl*-biotin, m. p. 231–233°, was obtained. A second crop of 0.3 g., melting at 230–232°, was obtained by concentrating the water solution; in this way the total recovery of pure material was 82% from the crude *dl*-biotin or 63% over-all yield from the crude *dl*-diamido ester.

Because of the limited solubility of *dl*-biotin in water, it was easier to crystallize it from a sufficient amount of dilute

ammonium hydroxide to effect solution followed by acidification. Thus, 5.9 g. of *dl*-biotin was dissolved in 50 cc. of dilute ammonium hydroxide. The solution was filtered and acidified to congo red with hydrochloric acid. The *dl*-biotin was removed by filtration, washed with water and dried; m. p. 232–233°; yield, 5.6 g. (95%). When the solution was acidified while hot, the *dl*-biotin crystallized in long needles.

(c) *dl-epi*-Allobiotin.—One and one-half grams of *dl-epi*-allodiamido acid, m. p. 189–190°, was hydrolyzed with barium hydroxide and treated with phosgene as described for *dl*-biotin. After one recrystallization from water, the yield of *dl-epi*allobiotin was only 0.2 g. (22%); this compound decomposed gradually above 195° without melting.

Anal. Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.23; H, 6.75; N, 11.21.

Preparation of Samples of the Diamido Acids or Esters for Microbiological Assay.—3,4-Dibenzamidotetrahydro-2-thiophenevaleric acid,[6] prepared from biotin, was used as a standard with which to compare the unknown diamido derivatives under the various hydrolysis conditions. In a typical experiment, 10 mg. of a crude fraction of *dl*-diamido ester was placed in a small Pyrex test-tube and to it was added 200 mg. of finely ground barium hydroxide octahydrate and 1 cc. of water. The tube was sealed and heated at 135–140° for sixteen hours. After opening the tube, the turbid solution and the two 0.5-cc. portions of wash water were transferred to a centrifuge tube. This solution was warmed in a hot water-bath and acidified to congo red with 1 *N* sulfuric acid. The barium sulfate was removed by centrifugation and washed with two 1-cc. portions of hot water. The combined solutions were made neutral with saturated aqueous sodium carbonate and about 1 cc. more saturated sodium carbonate solution was added. The solution was cooled in an ice-bath and treated with phosgene until it gave an acid reaction to congo red. It was then diluted to 10 cc. and suitable dilutions made for assay. The microbiological assay[11] showed 20% yield of biotin. Pure *dl*-diamido ester showed slightly better than 50% biotin activity compared to the standard by this procedure. The *dl*-allodiamido ester and the *dl-epi*-allodiamido ester showed no biotin activity.

Sodium hydroxide was tried also as the hydrolytic agent. About 5 mg. of the *d*-3,4-dibenzamido acid was placed in each of three small Pyrex test-tubes. To the samples was added respectively, 0.5–0.6 cc. of 1, 1.5 and 2 *N* aqueous sodium hydroxide. The sealed tubes were heated at about 140° for fifteen hours. The resulting solution from each tube was washed into a six-inch test tube. After cooling in ice, each sample was treated with phosgene until the solution was acid to congo red when silicic acid precipitated as a gel. Each sample was diluted to 10 cc. with water and an aliquot of 3 cc. was withdrawn, diluted to 10 cc. and filtered for assay. The assay showed the following per cent. recovery of biotin from the various hydrolyses: 22% from 1 *N* sodium hydroxide solution; 78% from both 1.5 and 2 *N* sodium hydroxide solution; 89% from the barium hydroxide hydrolysis described above.

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Summary

dl-Biotin and two stereochemically related racemates which are called *dl*-allobiotin and *dl-epi*-allobiotin have been synthesized from 4-benzamido-3-ketotetrahydrothiophene and methyl γ-formylbutyrate.

These two intermediates were condensed to yield 4-benzamido-3-keto-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid methyl ester. This unsaturated keto derivative was converted into two isomeric oximes which were reductively acetylated to yield two products: 3-acetamido-4-benzamido-4,5-dihydro-2-thiophenevaleric acid methyl ester and 3-acetamido-4-benzamido-$\Delta^{2,5}$-tetrahydro-2-thiophenevaleric acid methyl ester. These two products were hydrogenated over a palladium catalyst to yield three racemic forms of 3-acetamido-4-benzamido-tetrahydro-2-thiophenevaleric acid methyl ester. These three diamido esters were first hydrolyzed and then treated with phosgene to yield the three racemates stereochemically related to biotin.

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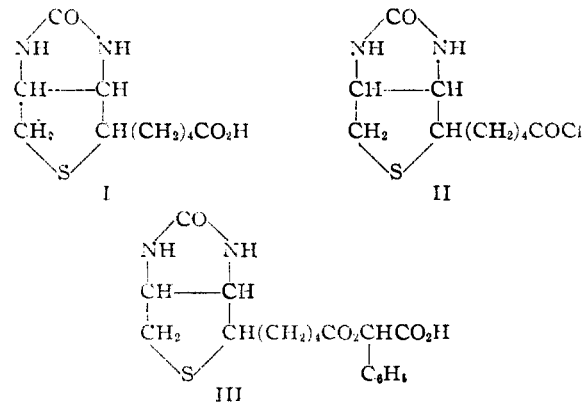
Biotin. VI. Resolution of *dl*-Biotin

BY DONALD E. WOLF, RALPH MOZINGO, STANTON A. HARRIS, R. CHRISTIAN ANDERSON AND KARL FOLKERS

Biotin was first obtained synthetically[1] by resolution and hydrolysis of the *d*(−)-mandelic acid esters of *dl*-biotin.[2,3] Later the resolution of the *l*(+)-arginine salts of *dl*-biotin gave biotin satisfactorily.

dl-Biotin (I) was converted to its acid chloride (II) with thionyl chloride and the acid chloride was allowed to react with *d*(−)-mandelic acid in chloroform solution to give the corresponding esters (III). The crude esters were then subjected to a series of fractional crystallizations



(1) Harris, Wolf, Mozingo and Folkers, *Science*, **97**, 447 (1943).

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