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BIOTIN. VI. SYNTHESIS OF BIOTIN AND *epi*ALLOBIOTIN

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Methods of synthesizing 2-(δ-carboxybutyl)thiophane-3,4-*trans*-dicarboxylic acid (I), an intermediate in the projected synthesis of biotin, have been the subject of some previous communications (1, 2). The conversion of this triacid to 2-(δ-carboxybutyl)-3,4-diaminothiophane involves the selective degradation of the two ring carboxyls, leaving the side chain carboxyl intact. This has been accomplished by degrading the carboxyl groups one at a time and proving the structure of the intermediates at each step in order to demonstrate that the proper carboxyl group had been converted to an amine.

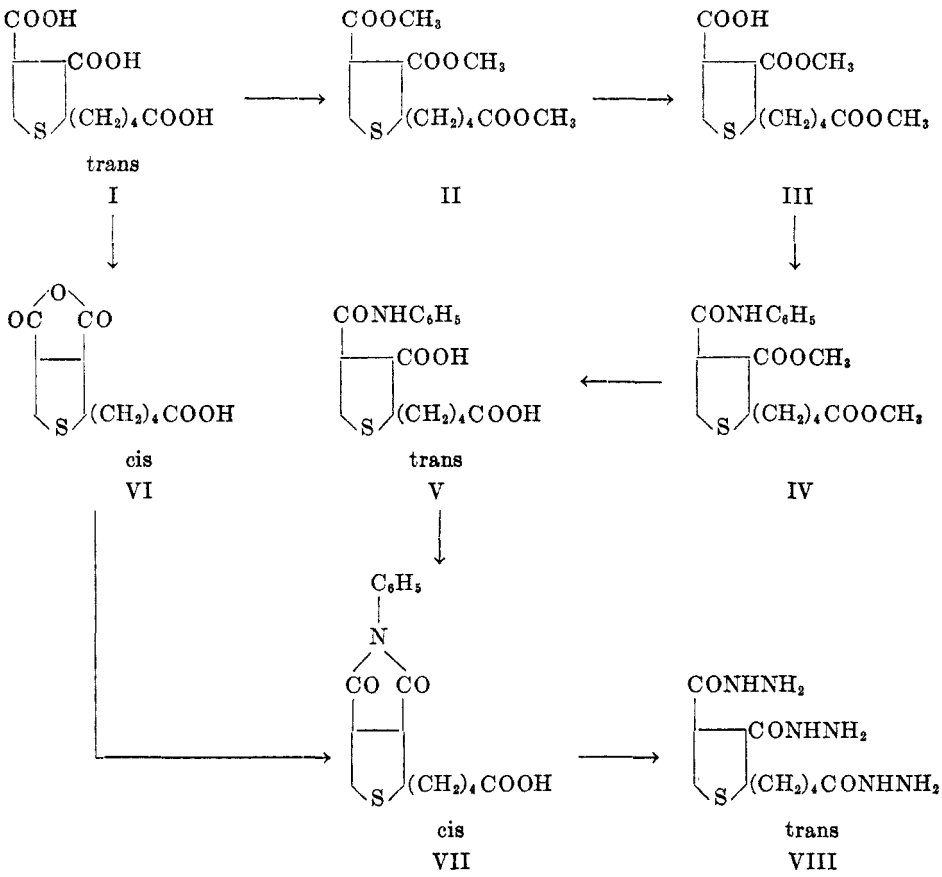
Treatment of the trimethyl ester (II) of I with one equivalent of alkali resulted in an oily diester monoacid, III, which gave a crystalline anilide, IV. If the side chain carboxyl group were the one that saponified, it would only be necessary to make a dihydrazide from the remaining two ester groups, then degrade them by the Curtius method. If one of the ring carboxyls saponified, then it would be necessary to degrade it *via* the acid chloride with sodium azide. That a free ring carboxyl group was present was proved in the following manner:

The diester, IV, was saponified to a carbanilido diacid, V. When this acid was boiled with propionic anhydride, inversion of configuration to *cis* and ring closure to 2-(δ-carboxybutyl)thiophane-3,4-*cis*-dicarboxanil (VII) took place. The structure was shown to be *cis* by treating 2-(δ-carboxybutyl)thiophane-3,4-*cis*-dicarboxylic anhydride (VI), prepared by inversion of the corresponding *trans* acid, with aniline followed by ring closure with acetyl chloride to the identical anil, VII.

As the two ring carboxyl groups in the anil, VII have been distinguished from the side chain free carboxyl group, it was considered possible that treatment with hydrazine would give 2-(δ-carboxybutyl)thiophane-3,4-*trans*-dicarboxhydrazide (3). However, under the conditions necessary to cleave the nitrogen ring, the side chain carboxyl group also surprisingly formed a hydrazide and the resultant product was the *trans*-trihydrazide (VIII). It was identified by comparison with the *trans*-trihydrazide prepared from the trimethyl ester (II).

As selective saponification of the triester took place on the ring, the free carboxyl in III was degraded by treating its acid chloride with sodium azide. The intermediate azide was rearranged to the isocyanate and condensed with aniline forming 2-(δ-carbomethoxybutyl)-3-carbomethoxy-*trans*-4-uranilinothiophane (IX). The crystalline ester was saponified to the corresponding *trans*-diacid, X. The position of the uranilino group was proved by hydrogenolysis of the 2-(δ-carboxybutyl)-4-uranilinothiophane-3-*trans*-carboxylic acid in 20% sodium hydroxide at 200–210° and 70 atmospheres of hydrogen in the presence of U.O.P. nickel catalyst. The product was α-ethylsuberic acid (XI), probably

formed by hydrogenolysis of the sulfur, hydrolysis of the uranilino group, deamination of the resultant amino acid and reduction of the double bond. It



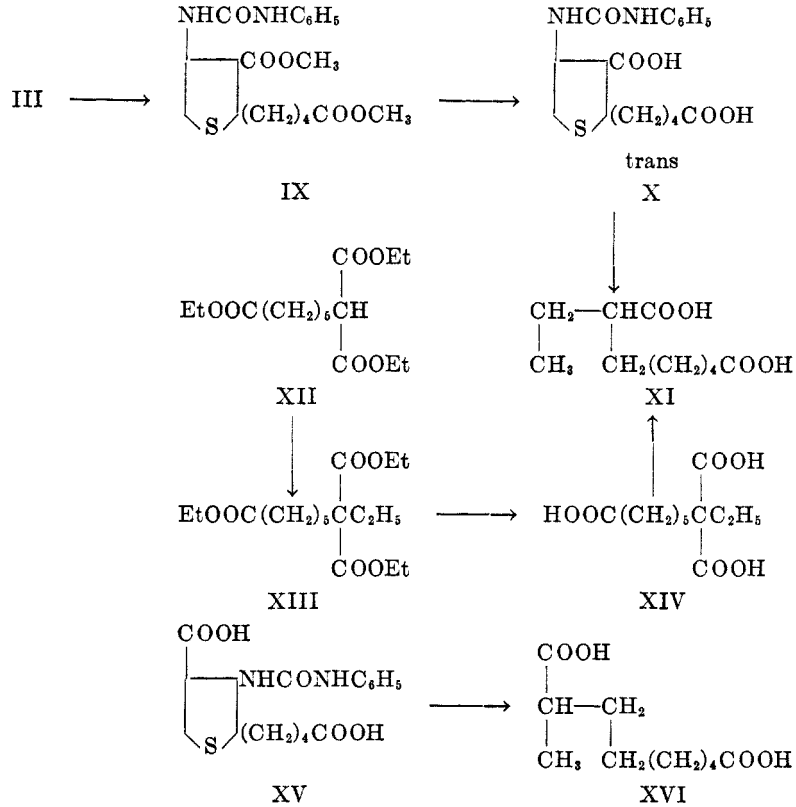
was identical with an authentic sample prepared from ethyl α-carbethoxysuberate as shown.

If the product of the Curtius degradation were the isomeric uranilino diacid, XV, then the hydrogenation product should have been α-methylazelaic acid (XVI).

A study of the selective hydrolysis of 2-(δ-carbomethoxybutyl)-3-carbomethoxy-*trans*-4-uranilinothiophane (IX) was made and although over twenty experiments were run, no clear-cut products could be obtained.

It was found possible to distinguish between the remaining two carboxyls by formation of 3-phenyl-6-(δ-carboxybutyl)-5,6,8,9-tetrahydrothieno[3,4,e,*cis*] uracil (XVII) by methods worked out on the model uracil without a side chain described in the previous communication (4). By refluxing a solution of 2-(δ-carboxybutyl)-4-uranilinothiophane-*trans*-3-carboxylic acid (X) with acetic anhydride and sodium acetate, followed by hydrolysis of the 1-acetyl group with dilute hydrochloric acid, the *cis*-uracil, XVII, was obtained. The intermediate

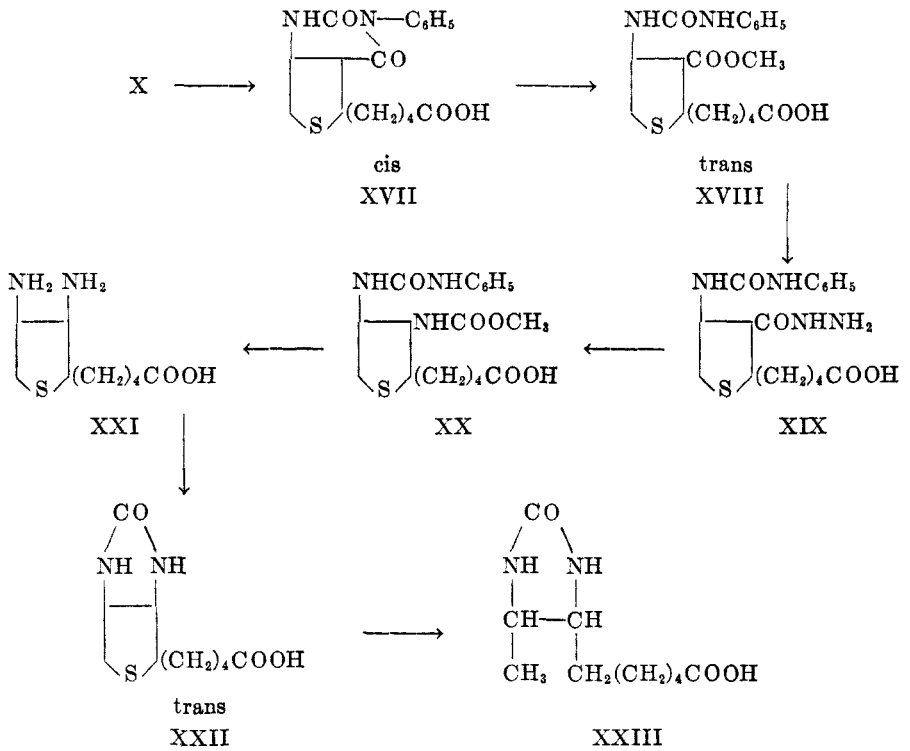
acetyl derivative could be isolated, but the over-all yield (63–76%) was much better if it was hydrolyzed without isolation. The nitrogen ring of the uracil



(XVII) on short boiling with methanolic sodium methoxide was ruptured with inversion of configuration to form 2-(δ-carboxybutyl)-3-carbomethoxy-*trans*-4-uranilinothiophane (XVIII) in 74% yield. The monoester was easily converted to the *trans* hydrazide, XIX. The configuration was established as *trans* in two ways. Hydrolysis of the hydrazide with 6 *N* hydrochloric acid gave the *trans*-uranilino diacid, X. If the configuration had been *cis*, the uracil, XVII, would have been reformed. The second method was by Curtius degradation of the hydrazide, which formed a *trans* urethan, XX, rather than 2-(δ-carboxybutyl)-5-keto-6-carbanilidoimidazolido[4,5,c,*cis*]thiophane (XXVIII) (4). The 2-(δ-carboxybutyl)-3-carbomethoxyamino-*trans*-4-uranilinothiophane (XX) was hydrolyzed to the diamine, XXI, with aqueous barium hydroxide at 150°, which was isolated as the sulfate, m.p. 272–275° dec. Treatment with phosgene in aqueous potassium carbonate caused the separation of a *trans*-biotin isomer, XXII, with no definite melting point, but gradually decomposing over 185°.

Harris *et al.* (5) have described *dl*-biotin and two of its isomers. They reported *dl*-biotin to melt at 232°, *dl*-allobiotin to melt at 194–196° and *dl-epi*allobiotin to decompose without melting starting at 195°. Thus the biotin isomer,

XXII, from its melting point, may be either *dl-epi*allobiotin or the unknown *dl-epi*biotin. This point was proved by desulfurization of XXII to the biologically

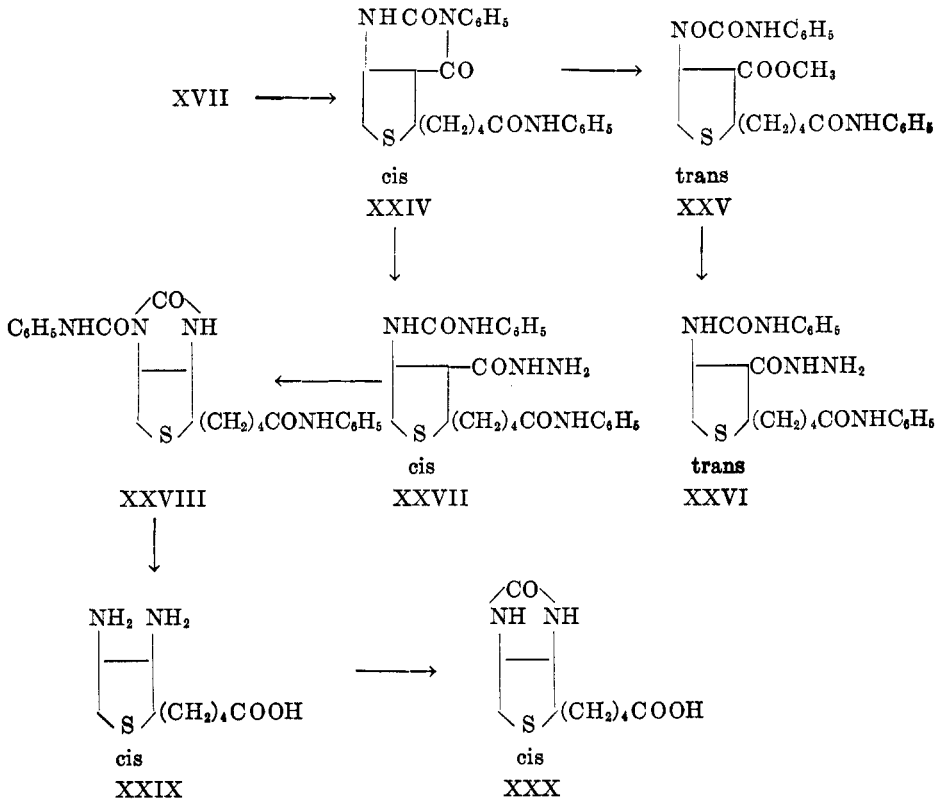


inactive *dl*-desthioallobiotin (XXIII) (5). If XXII were *dl-epi*biotin, then the biologically active *dl*-desthiobiotin (5) would be formed on desulfurization. Thus the *trans*-biotin isomer, XXII, is *dl-epi*allobiotin. Therefore biotin and *epi*-biotin must be *cis* isomers and allobiotin a *trans* isomer.[1]

When the uracil valeric acid (XVII) was treated with hydrazine, ring cleavage occurred with partial inversion to a mixture of *cis* and *trans* hydrazides (XIX). This difficulty was attributed to the ready solubility of a hydrazide with a free carboxyl group in hydrazine. When the carboxyl group of the uracil was blocked by conversion to its anilide, XXIV, then hydrazine smoothly opened the uracil ring in 95% yield to the insoluble *cis*-hydrazide, XXVII. The configuration was established as *cis* in two ways. When the uracil anilide, XXIV, was treated with methanolic sodium methoxide, the *trans* methyl ester, XXV, was formed which was readily converted to the *trans*-hydrazide, XXVI. A mixture of the *cis* and *trans* hydrazides gave a depression in melting point.

[1] Following the completion of this work, Harris and his co-workers (10) presented evidence that biotin was a derivative of a *cis*-diamine and that allobiotin and *epi*allobiotin were derived from *trans*-diamines. They also recorded (11) the m.p. of *dl-epi*allodiamine sulfate as 283–285° and *dl*-allodiamine sulfate as 228–230°. The m.p. of the former agrees with the m.p. of the sulfate of XXI.

The second proof of configuration was conversion of the *cis*-hydrazide to 2-(δ-carbanilidobutyl) - 5 - keto - 6 - carbanilidoimidazolido[4,5,c,*cis*]thiophane

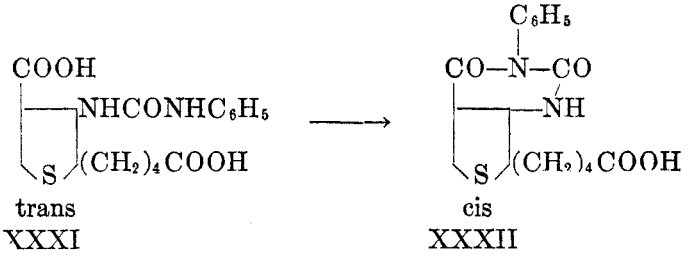


(XXVIII) on diazotization. This ring formation of the isocyanate is characteristic of the *cis* series (4). The diazotization of the *cis*-hydrazide, XXVII, in the usual manner with sodium nitrite in 50% methanol which was 0.5 *N* in hydrochloric acid gave only traces of an azide, most of the hydrazide being recovered unchanged. However, when the diazotization was run at 100° with butyl nitrite in dry butanol containing hydrogen chloride (6), a 55% yield of the imidazolone, XXVIII, was obtained. Hydrolysis to the *cis*-diamine sulfate, XXIX, was accomplished smoothly by long heating with barium hydroxide solution at 160°. Treatment of the diamine with phosgene gave *dl*-biotin, m.p. 230–232°, which was 50% as active as natural biotin when assayed against *L. arabinosis* or yeast.

It should be pointed out that this method of synthesis offers chemical control of isomers and no fractional crystallizations are necessary in order to separate the isomers. The average yield of *dl*-biotin from pimelic acid is of the order of 1.7% or 2.7 g. per 100 g. of pimelic acid.

If the carboxyl group in the 3 position of the crystalline *trans* isomer of 2-(δ-carboxybutyl)thiophane-3,4-dicarboxylic acid (I) could be degraded to the

3-uranilino acid, XXXI, and converted to a *cis* uracil, XXXII, then inversion would take place at C_4 and the configuration of *epi*biotin would be obtained.



Work towards the preparation of XXXII, and *epi*biotin has been completed and will be described in a future communication.

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EXPERIMENTAL

2-(δ-Carbomethoxybutyl)-3,4-trans-dicarbomethoxythiophane (II). A mixture of 20.6 g. of 2-(δ-carboxybutyl)thiophane-3,4-*trans*-dicarboxylic acid (I), 125 cc. of methanol, 175 cc. of chloroform, and 5 cc. of concentrated sulfuric acid was refluxed for sixteen hours under a Soxhlet apparatus containing anhydrous magnesium sulfate in the thimble. The solution was washed with several volumes of water containing excess sodium bicarbonate. Distillation of the chloroform left 22.5 g. (94%) of the triester as an oil. In other runs the yield was 89–94%.

Anal. Calc'd for $C_{14}H_{22}O_6S$: C, 52.8; H, 7.0.
Found: C, 52.9; H, 7.0.

2-(δ-Carbomethoxybutyl)-3-carbomethoxythiophane-4-carboxylic acid (III). To a solution of 22.5 g. of the above trimethyl ester in 110 cc. of methanol was added 31 cc. of 10% sodium hydroxide. After a minimum of sixteen hours at room temperature, the solution was concentrated to about one-half its volume, diluted with 100 cc. of water and 300 cc. of saturated sodium bicarbonate solution, and extracted with benzene. The benzene extract on evaporation gave 6.4 g. (24%) of unchanged triester. The alkaline solution was acidified and extracted with benzene. The extract, washed with dilute hydrochloric acid, was evaporated leaving 17.6 g. (69%) of crude product.

In other runs the yield of recovered triester was 20–30% and the yield of product 60–70%.

By saturation of the aqueous mother liquor with salt and extraction with ethyl acetate, the remainder of the triester could be accounted for as monoester diacids, which could be esterified and used again. As the latter are insoluble in benzene, satisfactory separation of the monoacid diesters from the monoester-diacids was readily accomplished.

2-(δ-Carbomethoxybutyl)-3-carbomethoxy-4-trans-carbanilidothiophane (IV). A solution of 5 g. of 2-(δ-carbomethoxybutyl)-3-carbomethoxythiophane-*trans*-4-carboxylic acid (III) and 5 cc. of thionyl chloride in 20 cc. of benzene was refluxed for fifteen minutes, when gas evolution was complete. The solvent was removed *in vacuo* (bath 45°) and the evaporation repeated with 25 cc. of benzene. The residue was dissolved in 50 cc. of benzene and treated with 6 cc. of aniline. After ten minutes the solution was washed twice with dilute hydrochloric acid, aqueous sodium bicarbonate and water. Solvent was evaporated and the residue crystallized from methanol; yield, 2.4 g. (38%), m.p. 116–119°. Repeated recrystallization from methanol failed to raise the m.p. above 118–124°.

Anal. Calc'd for $C_{19}H_{25}NO_5S$: C, 60.2; H, 6.6; N, 3.7.
Found: C, 60.2; H, 6.3; N, 4.2.

2-(δ-Carboxybutyl)-4-carbanilidothiophane-3-trans-carboxylic acid (V). To a solution of 3 g. of the above ester, IV, in 25 cc. of hot methanol was added 12 cc. of 10% sodium hydroxide. The solution was boiled down on the steam-bath for one hour, then diluted with 20 cc. of water, and acidified. The product was washed with water; yield 2.75 g. (98%), m.p. 238–242°. Recrystallization from alcohol gave white crystals, m.p. 253–255°.

Anal. Calc'd for $C_{17}H_{21}NO_5S$: C, 58.2; H, 6.0; N, 4.0.
Found: C, 58.2; H, 6.1; N, 3.6.

2-(δ-Carboxybutyl)thiophane-3,4-cis-dicarboxylic anhydride (VI). A mixture of 500 mg. of 2-(δ-carboxybutyl)thiophane-3,4-*trans*-dicarboxylic acid (I), 150 mg. of anhydrous sodium acetate and 5 cc. of acetic anhydride was refluxed for thirty minutes, then diluted with 5 cc. of water containing 0.3 cc. of concentrated hydrochloric acid. After the excess anhydride had decomposed, the solution was evaporated to dryness *in vacuo* and extracted with acetone. The acetone was concentrated to a small volume and diluted with benzene almost to turbidity. After standing overnight the crystals of *cis*-triacid were collected; yield, 240 mg. As the triacid appeared to be hygroscopic and difficult to handle it was converted to the anhydride by evaporative distillation at 190° (1 mm.). The crystalline distillate was purified by recrystallization from chloroform-petroleum ether, white crystals, m.p. 110–111°.

Anal. Calc'd for $C_{11}H_{14}O_5S$: C, 51.2; H, 5.5.
Found: C, 51.5; H, 6.2.

2-(δ-Carboxybutyl)thiophane-3,4-cis-dicarboxanil (VII). (*A*). A solution of 1.9 g. of 2-(δ-carboxybutyl)-4-carbanilidothiophane-3-*trans*-carboxylic acid (V) in 20 cc. of propionic anhydride was refluxed for two hours. After the cautious addition of 20 cc. of water, the solution was refluxed for fifteen minutes, then diluted to about 200 cc. with water. The product weighed 1.22 g. (68%), m.p. 134–136°. Recrystallization from water containing a little acetic acid raised the m.p. to 136–139°.

Anal. Calc'd for $C_{17}H_{19}NO_4S$: C, 61.3; H, 5.8; N, 4.2.
Found: C, 61.2; H, 6.1; N, 4.8.

(*B*). To a solution of 100 mg. of 2-(δ-carboxybutyl)thiophane-3,4-*cis*-dicarboxylic anhydride (VI) in 5 cc. of hot chloroform was added 0.1 cc. of aniline. A gummy anilic acid immediately separated which could not be crystallized, indicating that ring opening had taken place in two ways. After the addition of 10 cc. of benzene, the solvent was decanted from the anilic acid. The latter was cyclized by refluxing its solution in 10 cc. of acetyl chloride for fifteen minutes. The acetyl chloride was evaporated and the oily residue was heated on the steam-bath with 4 cc. of 50% acetic acid for ten minutes, then diluted to 25 cc. with water; yield, 60 mg. (46%), m.p. and mixed m.p. with preparation *A*, 136–138°.

Trihydrazide of 2-(δ-carboxybutyl)thiophane-3,4-trans-dicarboxylic acid (VIII). A solution of 1.05 g. of 2-(δ-carboxybutyl)thiophane-3,4-*cis*-dicarboxanil (VII) in 2.5 cc. of 100% hydrazine hydrate was heated on the steam-bath under reflux for fourteen hours, then dissolved in 20 cc. of methanol. The product soon crystallized from the solution; yield, 0.36 g. (38%), m.p. 218–220°. The trihydrazide is readily soluble in water.

Anal. Calc'd for $C_{11}H_{22}N_6O_3S$: N, 26.4. Found: N, 26.4.

The methanol filtrate was evaporated to dryness and treated with 2 cc. of hydrazine again. An additional 0.23 g. (total, 62%) of product was obtained, m.p. 215–217°.

The tribenzylidine derivative formed rapidly in hot dilute alcohol in 98% yield. It was purified by recrystallization from Methyl Cellosolve, white crystals, m.p. 212–214°.

Anal. Calc'd for $C_{32}H_{34}N_6O_3S$: N, 14.5. Found: N, 14.5.

This compound was insoluble in hot aqueous sodium bicarbonate or 3% alkali.

The reaction was also run on the anil, VII, with hydrazine containing one equivalent of sodium hydroxide. The trihydrazide was again isolated. The filtrate was treated with benzaldehyde, but no water-insoluble, alkali-soluble dibenzylidine derivative was formed.

2-(δ-Carboxybutyl)-4-uranilinothiophane-trans-3-carboxylic acid (X) and dimethyl ester (IX). (*A*). A mixture of 11.8 g. of crude 2-(δ-carbomethoxybutyl)-3-carbomethoxythiophane-4-*trans*-carboxylic acid (III), 12 cc. of thionyl chloride and 50 cc. of benzene was

refluxed for fifteen minutes, then evaporated to dryness *in vacuo* (bath 50°). After the addition of 25 cc. of benzene, the evaporation was repeated. The residual acid chloride, cooled in an ice-bath, was dissolved in 50 cc. of acetone and added dropwise with stirring and ice cooling to a solution of 3.1 g. of sodium azide in 50 cc. of water at such a rate that the temperature was just below 10°. After being stirred one hour at 0°, the mixture was diluted with several volumes of water and the oily azide extracted twice with chloroform. The combined extracts, after being dried with calcium chloride at 0°, were refluxed for thirty minutes, treated with 5 cc. of aniline and refluxed five minutes longer. Washed with dilute hydrochloric acid, the solution was evaporated to dryness and the residue crystallized from 60 cc. of methanol at 0°. The dimethyl ester (IX) was washed with ice cold methanol; yield, 6 g. (39%) of crystals, m.p. 124–128°.

Anal. Calc'd for $C_{19}H_{26}N_2O_5S$: N, 7.1. Found: N, 7.2.

The combined filtrate and washings were diluted with 25 cc. of 10% sodium hydroxide and boiled on the steam-bath for one hour, then diluted with water and clarified with Norit. Acidification gave a gummy precipitate which was suspended in ethyl acetate and shaken or stirred until the lumps had disintegrated. After standing overnight to complete crystallization, the diacid was washed with ethyl acetate; yield, 2.7 g. (19%) of product, m.p. 188° dec.

In other runs the diester was not isolated, but the mixture hydrolyzed directly. The yield of diacid was 40–45%.

(*B*) To a hot solution of 2.6 g. of 2-(δ-carbomethoxybutyl)-3-carbomethoxy-*trans*-4-uranilinothiophane (IX) in 50 cc. of methanol was added 15 cc. of 10% sodium hydroxide solution. After being boiled on the steam-bath for one hour, during which time most of the methanol evaporated, the solution was diluted with water, clarified with Norit, and acidified. The diacid, X, weighed 2.2 g. (92%), m.p. 182–183° dec. It was purified by recrystallization from dilute alcohol, white crystals, m.p. 190–191° dec.

Anal. Calc'd for $C_{17}H_{22}N_2O_5S$: C, 55.7; H, 6.1; N, 7.7.
Found: C, 55.9; H, 6.2; N, 8.0.

α-Ethyl-α-carboxysuberic acid (XIV). Ethyl α-carbethoxysuberate (XII) was prepared in 87% yield essentially according to procedure of Wood and duVigneaud (7) except that 1:2 absolute alcohol-benzene was used as a solvent and a little sodium iodide was added as a catalyst.

To a solution of 1.6 g. of sodium methoxide (Mathieson Alkali Works) in 10 cc. of absolute alcohol was added 8 g. of ethyl α-carbethoxysuberate and 4 cc. of ethyl iodide. After being refluxed for fifteen hours, the solution was acidified with acetic acid and most of the alcohol evaporated. The residue was diluted with water containing some sodium bisulfite and extracted with benzene. The benzene extract was washed with water and evaporated. Saponification of the ester with 9 g. of potassium hydroxide in 18 cc. of water diluted with 20 cc. of alcohol was complete after the mixture was heated on the steam-bath for three hours. The cooled solution, diluted with 25 cc. of water was acidified. After standing overnight the product was washed with water; yield, 4.6 g. (65%), m.p. 155–156° dec. A sample was recrystallized from ethyl acetate, white crystals, m.p. 155–156° dec.

Anal. Calc'd for $C_{11}H_{18}O_6$: C, 53.7; H, 7.4.
Found: C, 54.1; H, 7.7.

α-Ethylsuberic acid (XI). A mixture of 4.1 g. of α-ethyl-α-carboxysuberic acid and a trace of copper oxide was heated at 175–180° for fifteen minutes when gas evolution was complete. The crystalline residue obtained on cooling was purified by recrystallization from benzene-heptane with the aid of Norit; yield, 2.9 g. (87%), m.p. 74–75°.

Anal. Calc'd for $C_{10}H_{18}O_4$: C, 59.3; H, 9.0.
Found: C, 58.9; H, 9.3.

The *p*-phenylphenacyl ester, prepared according to Shriner and Fuson (8), formed white crystals from methanol-acetone, m.p. 80–81°.

Anal. Calc'd for $C_{38}H_{38}O_6$: C, 77.2; H, 6.5.
Found: C, 77.2; H, 6.1.

The S-benzyl isothiouronium salt was prepared in water and recrystallized from isopropyl alcohol; white crystals, m.p. 145–146°.

Anal. Calc'd for $C_{26}H_{38}N_4O_4S_2$: C, 58.3; H, 7.2; N, 10.5.
Found: C, 58.6; H, 7.7; N, 10.3.

Hydrogenolysis of 2-(δ-carboxybutyl)-4-uranilinothiophane-3-trans-carboxylic acid (X). A mixture of 450 mg. of the uranilino acid (X), 4.5 g. of pulverized U.O.P. nickel catalyst (Universal Oil Products Co.), and 10 cc. of 20% sodium hydroxide was shaken with hydrogen at 70 atm. and 200–210° for twenty-two hours. The bomb contents were rinsed out with 200 cc. of 1% sodium hydroxide and the mixture heated on the steam-bath with occasional mixing for twenty minutes. The catalyst was removed by filtration through Celite and washed with water. The filtrate was acidified and extracted with ethyl acetate. Removal of the solvent left an oil which rapidly evaporatively distilled at 150° (1 mm.) and partially solidified on the cold finger; yield, 100 mg.

The crude *p*-phenylphenacyl ester was an oil which crystallized on seeding with the *p*-phenylphenacyl ester of α-ethylsuberic acid. It was purified by recrystallization from methanol-acetone, white crystals, m.p. 79–81°. A mixture with the *p*-phenylphenacyl ester of α-ethylsuberic acid melted at 80–81°.

The S-benzyl isothiouronium salt was prepared in water and recrystallized from isopropyl alcohol; white crystals, m.p. 145–146°. A mixture with the S-benzyl isothiouronium salt of α-ethylsuberic acid gave no depression in m.p.

Anal. Calc'd for $C_{26}H_{38}N_4O_4S$: N, 10.5. Found: N, 10.4.

3-Phenyl-6-(δ-carboxybutyl)-5,6,8,9-tetrahydrothieno[3,4,e,cis]uracil (XVII). A mixture of 6.3 g. of 2-(δ-carboxybutyl)-4-uranilinothiophane-*trans*-3-carboxylic acid (X), 6.3 g. of sodium acetate and 130 cc. of acetic anhydride was refluxed in an oil-bath for one hour, then diluted cautiously with 65 cc. of water. After the acetic anhydride had decomposed, the solution was diluted with 60 cc. of concentrated hydrochloric acid and refluxed thirty minutes. The hot solution was clarified with Norit, using 50% acetic acid as a wash, then evaporated to a paste *in vacuo*, diluted with water and the product collected on a filter; yield, 3.8 g. (63%), m.p. 191° dec.

In other runs the yields were 63–76% probably depending upon the purity of the starting material. For analysis a sample was recrystallized from dilute methanol, white crystals, m.p. 202–203°.

Anal. Calc'd for $C_{17}H_{20}N_2O_4S$: C, 58.7; H, 5.8; N, 8.0.
Found: C, 58.5; H, 6.3; N, 7.6.

The 1-acetyl derivative can be isolated in 42% yield, m.p. 186–194°, by eliminating the hydrochloric acid and second heating period. Recrystallization from dilute alcohol gave white crystals, m.p. 191–195°.

Anal. Calc'd for $C_{19}H_{22}N_2O_5S$: C, 58.4; H, 5.7.
Found: C, 58.5; H, 5.4.

2-(δ-Carboxybutyl)-3-carbomethoxy-trans-4-uranilinothiophane (XVIII). To a boiling mixture of 1.0 g. of XVII and 40 cc. of methanol was added a solution of 120 mg. of sodium in 8 cc. of methanol. After being boiled on the steam-bath for fifteen minutes, the solution was acidified with 0.8 cc. of acetic acid, diluted with water until turbid, and cooled in an ice-bath; yield, 0.80 g. (74%), m.p. 134–135°. Recrystallization from dilute methanol did not raise the m.p.

Anal. Calc'd for $C_{18}H_{24}N_2O_5S$: C, 56.8; H, 6.4; N, 7.4.
Found: C, 56.5; H, 6.5; N, 7.7.

The ester was hydrolyzed in quantitative yield by heating one hour with 3 *N* hydrochloric acid in 50% acetic acid to 2-(δ-carboxybutyl)-4-uranilinothiophane-*trans*-3-carboxylic acid (X) and identified by mixed m.p.

2-(δ-Carboxybutyl)-4-uranilinothiophane-trans-3-carboxhydrazide (XIX). A solution of 500 mg. of 2-(δ-carboxybutyl)-3-carbomethoxy-*trans*-4-uranilinothiophane (XVIII) in 1 cc. of 100% hydrazine hydrate was heated on the steam-bath for fifteen minutes, diluted with water and acidified with acetic acid. The somewhat gelatinous product was collected on a

filter, washed with water, and dried on the steam-bath; yield, 450 mg. (90%) of white solid, m.p. 223° dec.

Anal. Calc'd for $C_{17}H_{24}N_4O_4S$: C, 53.7; H, 6.4; N, 14.7.
Found: C, 53.6; H, 6.6; N, 14.2.

When 20 mg. of this hydrazide was heated with 1 cc. of 6 *N* hydrochloric acid and 1 cc. of acetic acid for one hour, 2-(δ-carboxybutyl)-4-uranilinothiophane-3-*trans*-carboxylic acid (X) crystallized on cooling, m.p. and mixed m.p. 181°. A mixture with 3-phenyl-6-(δ-carboxybutyl)-5,6,8,9-tetrahydrothieno[3,4,e,*cis*]uracil (XVII) gave a depression in m.p.

2-(δ-Carboxybutyl)-3-carbomethoxyamino-4-trans-uranilinothiophane (XX). A mixture of 660 mg. of the above hydrazide (XIX), 51 cc. of methanol and 51 cc. of 0.33 *N* hydrochloric acid was heated until solution took place, then cooled in an ice-bath. A solution of 150 mg. of sodium nitrite in 10 cc. of water was added dropwise with stirring over a period of ten minutes. After being stirred an additional ten minutes at 0°, the mixture was diluted with ice-water and the azide extracted with ethyl acetate. The extract, dried with magnesium sulfate at 0°, was diluted with one-half volume of methanol and refluxed for thirty minutes. Solvent was evaporated and the residue triturated with ethyl acetate; yield 430 mg. (63%), m.p. 203–204° dec. Recrystallization from methanol did not change the m.p.

Anal. Calc'd for $C_{18}H_{25}N_3O_5S$: C, 54.7; H, 6.4; N, 10.3.
Found: C, 54.9; H, 7.0; N, 10.3.

2-(δ-Carboxybutyl)-trans-3,4-diaminothiophane (XXI) sulfate (dl-epiallodiamine sulfate). A mixture of 430 mg. of 2-(δ-carboxybutyl)-3-carbomethoxyamino-*trans*-4-uranilinothiophane (XX), 4 g. of barium hydroxide, and 10 cc. of water was shaken in a bomb at 150° for twenty hours. The bomb contents were rinsed out with water and excess baryta removed with dry ice. The filtrate from the barium carbonate was washed three times with chloroform to remove aniline, then made just acidic to Congo Red with 1 *N* sulfuric acid. After removal of the barium sulfate the solution was evaporated to dryness *in vacuo* and the residue triturated with methanol; yield, 210 mg. (61%) of white crystals which decomposed between 250–260°. Recrystallization from water raised the m.p. to 272–275° dec.

Anal. Calc'd for $C_9H_{20}N_2O_6S_2$: C, 34.2; H, 6.4.
Found: C, 33.6; H, 6.7.

Harris, *et al.* (11) give the m.p. of *dl-epi*allodiamine sulfate as 283–285° and *dl*-allodiamine sulfate as 228–230°.

2-(δ-Carboxybutyl)-5-ketoimidazolido[4,5,c,trans]thiophane (XXII) (dl-epiallobiotin). A solution of 900 mg. of 2-(δ-carboxybutyl)-3,4-*trans*-diaminothiophane (XXI) sulfate in 120 cc. of 10% potassium carbonate solution was treated with phosgene at 0° until acidic. The product was washed with ice-water; yield, 450 mg. (65%) of white crystals which gradually decompose over 185°. On recrystallization from 50% methanol it formed long white needles which had the same m.p. However, when plunged into a block at 215°, it melted with decomposition in forty seconds.

Anal. Calc'd for $C_{10}H_{16}N_2O_3S$: C, 49.2; H, 6.6; N, 11.5.
Found: C, 48.9, 48.7; H, 7.3, 6.7; N, 11.5.

The methyl ester, prepared from the acid in methanol with ethereal diazomethane, formed white needles from dilute methanol, micro m.p. 150–151°.

Anal. Calc'd for $C_{11}H_{18}N_2O_3S$: C, 51.2; H, 7.0; N, 10.8.
Found: C, 51.4; H, 7.5; N, 11.0.

The sulfur was removed from the acid by hydrogenolysis with Raney nickel at room temperature in dilute aqueous sodium bicarbonate. The product, *dl*-desthioallobiotin (XXIII) was purified by recrystallization from water, m.p. 163–165°. This compound had no biotin activity when assayed by the yeast method.

Anal. Calc'd for $C_{10}H_{18}N_2O_3$: N, 13.1. Found: N, 12.8.

Harris, *et al.* (5) give the m.p. of *dl-epi*allobiotin as gradually decomposing over 195° and *dl*-desthioallobiotin as 165–166°. They reported that the latter was inactive for the growth of yeast.

3-Phenyl-6-(δ-carbanilidobutyl)-5,6,8,9-tetrahydrothieno[3,4,e,cis]uracil (*XXIV*). A mixture of 3 g. of 3-phenyl-6-(δ-carboxybutyl)-5,6,8,9-tetrahydrothieno[3,4,e,*cis*]uracil (XVII), 12 cc. of dry ether containing 0.5% pyridine and 24 cc. of thionyl chloride was shaken at room temperature until solution was complete (fifteen minutes). The solution was evaporated to dryness *in vacuo* (bath 40°). The residual, partially crystalline, acid chloride was suspended in 150 cc. of acetone at 0°, 7.8 cc. of aniline was added and the mixture shaken until most of the acid chloride had dissolved. The reaction was then finished by slight warming on the steam-bath. The solution was diluted with 60 cc. of water, 7.8 cc. of concentrated hydrochloric acid was added, then the solution was diluted to about 900 cc. with water. The solid had m.p. 200–203°; yield, 3.55 g. (95%). A sample was purified for analysis by recrystallization from dilute acetone, white crystals, m.p. 212–213°.

Anal. Calc'd for $C_{23}H_{25}N_3O_3S$: C, 65.2; H, 6.0; N, 9.9.
Found: C, 65.1; H, 6.0; N, 9.5.

2-(δ-Carbanilidobutyl)-4-uranilinothiophane-cis-3-carboxhydrazide (*XXVII*). A mixture of 0.50 g. of XXIV and 4 cc. of 100% hydrazine hydrate was heated on the steam-bath with mixing for twenty minutes. At no time was solution complete, but the mixture became more pasty as the reaction proceeded. After dilution with water, the white solid was collected and dried on the steam-bath, m.p. 210–212° dec.; yield, 510 mg. (95%).

Anal. Calc'd for $C_{23}H_{29}N_5O_3S$: C, 60.7; H, 6.4.
Found: C, 61.1; H, 6.2.

If the uracil valeric acid, XVII, was treated with hydrazine in the same manner, it appeared that the product was a mixture of *cis* and *trans* hydrazides, probably due to the solubility of the product in the reagent.

2-(δ-Carbanilidobutyl)-3-carbomethoxy-4-trans-uranilinothiophane (*XXV*). (*A*). To a boiling suspension of 100 mg. of XXIV in 5 cc. of methanol was added 20 mg. of sodium methoxide in 1 cc. of methanol. The mixture rapidly became homogeneous. After being boiled for five minutes, the solution was acidified with acetic acid and diluted to about 25 cc. with water; yield, 98 mg. (91%), m.p. 171–174°. Recrystallization from chloroform-methanol gave white crystals, m.p. 187–190°.

Anal. Calc'd for $C_{24}H_{29}N_3O_4S$: C, 63.3; H, 6.4.
Found: C, 63.1; H, 6.6.

(*B*). A mixture of 250 mg. of 2-(δ-carboxybutyl)-3-carbomethoxy-4-*trans*-uranilinothiophane (XVIII), 1 cc. of thionyl chloride, and 2 cc. of a solution of one drop of pyridine in 5 cc. of dry ether was shaken occasionally for fifteen minutes at room temperature. The solution was evaporated to dryness *in vacuo* (bath 40°), the residue dissolved in 3 cc. of acetone and treated with 0.6 cc. of aniline. After five minutes the solution was diluted with water containing 1 cc. of concentrated hydrochloric acid; yield, 280 mg. (93%), m.p. 170–175°. Recrystallization from methanol raised the m.p. to 186–188°. A mixture with preparation *A* gave no depression in m.p.

2-(δ-Carbanilidobutyl)-4-uranilinothiophane-3-trans-carboxhydrazide (*XXVI*). A mixture of 100 mg. of 2-(δ-carbanilidobutyl)-3-carbomethoxy-*trans*-4-uranilinothiophane (XXV) and 0.8 cc. of hydrazine hydrate was heated on the steam-bath with mixing for twenty minutes. At no time did solution take place, but the mixture became more pasty as the reaction proceeded. The mixture was diluted with water and the hydrazide removed; yield, 85 mg. (85%) of white crystals, m.p. 238–239° dec.

Anal. Calc'd for $C_{23}H_{29}N_5O_3S$: C, 60.7; H, 6.3.
Found: C, 61.0; H, 6.3.

A mixture with the corresponding *cis*-hydrazide (XVII, m.p. 210–212° dec.) melted at 196–197° dec.

2-(δ-Carbanilidobutyl)-5-keto-6-carbanilidoimidazolido[4,5,c,cis]thiophane (*XXVIII*). To a hot suspension of 3.7 g. of 2-(δ-carbanilidobutyl)-4-uranilinothiophane-3-*cis*-carboxhydrazide (XXVII) in 125 cc. of dry butanol was added a solution of 0.42 g. of hydrogen chloride in 5 cc. of butanol. To the clear solution was added 1.04 cc. of butyl nitrite with

swirling. The solution was then heated on the steam-bath for one hour, gas evolution being complete in five minutes. Solvent was removed *in vacuo*, the residue cooled and triturated with methanol; yield, 1.83 g. (51%), m.p. 198–205°. From the filtrate an additional 0.26 g. (7%), m.p. about 180° was obtained.

A similar preparation was purified for analysis by recrystallization from methanol; white crystals, m.p. 214–216°.

Anal. Calc'd for $C_{23}H_{26}N_4O_3S$: C, 63.0; H, 6.0; N, 12.8.
Found: C, 63.0; H, 6.3; N, 12.6.

Attempts to carry out the Curtius degradation in the usual manner as described for the *trans* hydrazide, XIX, gave only traces of product which could not be isolated, but could be detected by hydrolysis of the mixture, treatment with phosgene, and bioassay of the resultant solution.

2-(δ-Carboxybutyl)-3,4-cis-diaminothiophane (XXIX) sulfate. A mixture of 17 g. of 2-(δ-carbanilidobutyl)-5-keto-6-carbanilidoimidazolido[4,5,c,cis]thiophane (XXVIII), 85 cc. of 50% methanol, and 68 g. of barium hydroxide octahydrate was heated and shaken in a bomb at 160° for forty-five hours. The bomb contents were rinsed out with water and the excess baryta removed with dry ice. The filtrate, washed three times with chloroform to remove aniline, was made just blue to Congo Red by the addition of 1 *N* sulfuric acid. After removal of the barium sulfate the solution was evaporated to dryness *in vacuo* and triturated with methanol. The almost colorless crystals were washed with methanol, m.p. 254–256° dec.; yield, 10.1 g. (83%).

For analysis a sample was recrystallized by solution in a small amount of water and addition of several volumes of methanol, white crystals, m.p. 257–258° dec.

Anal. Calc'd for $C_9H_{20}N_2O_6S_2$: C, 34.2; H, 6.4; N, 8.9; SO_4-S, 10.1.
Found: C, 34.9; H, 6.7; N, 8.6; SO_4-S, 9.9.

2-(δ-Carboxybutyl)-5-ketoimidazolido[4,5,c,cis]thiophane (dl-biotin) (XXX). A clarified solution of 650 mg. of 2-(δ-carboxybutyl)-3,4-*cis*-diaminothiophane (XXIX) sulfate in 26 cc. of 10% potassium carbonate was treated with phosgene with shaking and ice cooling until acidic. The white crystals which separated were washed with ice-water, m.p. 223–224°; yield 450 mg. (90%). Recrystallization from 50% aqueous methanol gave long white needles, m.p. 226–228°, micro m.p. 230–232°. An assay with *Lactobacillus arabinosis* showed that this compound had 52% of the activity of natural biotin.

Anal. Calc'd for $C_{10}H_{16}N_2O_3S$: C, 49.2; H, 6.6; N, 11.5.
Found: C, 49.1; H, 6.7; N, 11.2.

The methyl ester formed white crystals from methanol-ether, micro m.p. 127–128°. After this work was completed Grüssner, Bourquin, and Schnider (12) recorded the m.p. 130° for this compound.

Anal. Calc'd for $C_{11}H_{18}N_2O_3S$: C, 51.2; H, 7.0.
Found: C, 51.5; H, 7.1.

Harris, *et al.* (5) give the m.p. of *dl*-biotin as 232°.

Resolution with *l*(+)-arginine (9) gave biotin, micro m.p. 231–232°, $[\alpha]_D^{25}$ +89.7° (c = 1.00 in 0.1 *N* sodium hydroxide). Admixture with natural biotin gave no depression in the melting point.

SUMMARY

Controlled isomer syntheses of *dl*-biotin and *dl-epi*allobiotin have been described.

The two rings of biotin and *epi*biotin have been established to have a *cis* configuration at the bridgehead whereas those of *epi*allobiotin and allobiotin are related *trans*.

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