

$\beta$ -Piperidinoethyl 1-*p*-isopropylphenylcyclohexanecarboxylate hydrochloride melted at 194–195° after recrystallization from water.

*Anal.* Calcd. for  $C_{23}H_{36}O_2NCl$ : N, 3.56. Found: N, 3.45.

$\gamma$ - $\alpha$ -Naphthyl- $\gamma$ -cyanopimelonitrile was obtained in 55% yield by the cyanoethylation of  $\alpha$ -naphthylacetonitrile. It melted at 103–104.5° on recrystallization from methanol.

*Anal.* Calcd. for  $C_{18}H_{15}N_3$ : C, 79.09; H, 5.53. Found: C, 79.65; H, 5.53.

$\gamma$ - $\alpha$ -Naphthyl- $\gamma$ -carbamympimelic Acid.—Saponification of the trinitrile in alcoholic alkali resulted in the formation of the diacid amide which melted at 214–215° on recrystallization from water. All attempts at further hydrolysis or saponification were unavailing.

*Anal.* Calcd. for  $C_{18}H_{19}O_5N$ : C, 65.64; H, 5.81; N,

4.25; neut. equiv., 164.7. Found: C, 65.33; H, 6.24; N, 4.17; neut. equiv., 162.0.

We wish to thank Mr. A. Lanzilotti for technical assistance in this investigation and Miss Edith Sozio for assistance in preparation of the manuscript.

### Summary

1. A general synthesis of 1-arylcyclohexanecarboxylic acids has been described.

2. Certain esters of this general type show pronounced antispasmodic and some analgesic activity.

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

## The Synthesis of Homodesthiobiotin and Related Compounds

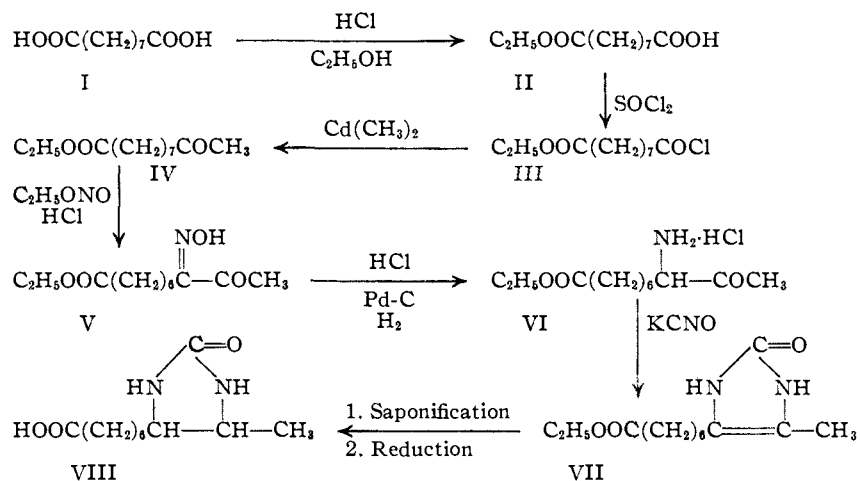
BY HERBERT MCKENNIS, JR.,<sup>1</sup> AND VINCENT DU VIGNEAUD<sup>2,3</sup>

Previous communications<sup>4,5</sup> have described the preparation of desthiobiotin, 5-methyl-2-oxo-4-imidazolidinecaproic acid, and its methyl ester by hydrogenolysis of natural biotin in the presence of Raney nickel catalyst. In addition, the total synthesis of desthiobiotin from  $\alpha$ -aminosuberic acid<sup>6</sup> as well as the resynthesis of desthiobiotin from  $\zeta$ , $\eta$ -diaminopelargonic acid<sup>7</sup> have been described.

In a continuation of the studies on the relation of the structure of biotin and desthiobiotin to biological activity, a number of related compounds including 5-methyl-2-oxo-4-imidazolidineheptanoic acid, *homodesthiobiotin*, were desired.

Although it appeared probable that the methods of synthesis which had been employed in this Laboratory for the synthesis of desthiobiotin<sup>6,7</sup> and related compounds<sup>8</sup> could be adapted to the

synthesis of homodesthiobiotin, a consideration of the availability of starting materials and the labor involved in the preparation of intermediates has led us to the selection of a previously unexplored route.



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(2) The authors wish to express their appreciation to Lederle Laboratories for a research grant which has aided greatly in this work.

(3) The authors also wish to thank Dr. Julian R. Rachele and Mr. R. C. Funk, Jr., of this Laboratory for carrying out the microanalyses, and Dr. Karl Dittmer, Mrs. Glenn Ellis, Miss Rachel Jewett and Miss Martha Fuchs for microbiological assays, the results of which will be presented in full elsewhere.

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

(5) Melville, Dittmer, Brown and du Vigneaud, *Science*, **94**, 308 (1941).

(6) Wood and du Vigneaud, *THIS JOURNAL*, **67**, 210 (1945).

(7) Melville, *ibid.*, **66**, 1422 (1944).

(8) Dittmer and du Vigneaud, *Science*, **100**, 129 (1944).

Gilman and Nelson<sup>9</sup> have developed the reaction of dialkyl cadmium compounds with acid chlorides as a method for the preparation of ketones. This reaction has been applied to the preparation of ethyl 6-oxoheptanoate from  $\delta$ -carboxyvaleryl chloride.<sup>10</sup> In view of the convenience of this method and the current availability of azelaic acid, a synthetic route to homodesthiobiotin with azelaic acid as the starting material was chosen. The steps in the synthesis are shown in the accompanying reactions.

Ethyl 9-oxodecanoate (IV) was prepared from  $\eta$ -carboxycaprylyl chloride (III) and dimethylcadmium. The keto ester was then treated with

(9) Gilman and Nelson, *Rec. trav. chim.*, **55**, 518 (1936).

(10) Cason and Prout, *THIS JOURNAL*, **66**, 46 (1944).

TABLE I  
 IMIDAZOLONE ESTERS

Compound	M. p., °C.	Formula	Analyses, %
Ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazolecarboxylate <sup>a</sup>	220-221	C <sub>7</sub> H <sub>10</sub> O <sub>3</sub> N <sub>2</sub>	
Ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazoleacetate	249-250	C <sub>8</sub> H <sub>12</sub> O <sub>3</sub> N <sub>2</sub>	Calcd.: N, 15.21; OC <sub>2</sub> H <sub>5</sub> , 24.5 Found: N, 15.04; OC <sub>2</sub> H <sub>5</sub> , 24.1
Ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazolebutyrate	218-219	C <sub>10</sub> H <sub>18</sub> O <sub>3</sub> N <sub>2</sub>	Calcd.: C, 56.63; H, 7.60 Found: C, 56.47; H, 7.35
Ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazolecaproate	194-195 <sup>b</sup>	C <sub>12</sub> H <sub>20</sub> O <sub>3</sub> N <sub>2</sub>	Calcd.: C, 59.97; H, 8.39; N, 11.66 Found: C, 60.15; H, 8.58; N, 11.42
Ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazoleheptanoate <sup>c</sup> (VII)	186-189 <sup>b</sup>	C <sub>13</sub> H <sub>22</sub> O <sub>3</sub> N <sub>2</sub>	.....

<sup>a</sup> References 12, 13 and 14. <sup>b</sup> The melting point varied considerably with the rate of heating. The value recorded was obtained by heating the material at a rate of 3° per minute on a micro melting point apparatus. <sup>c</sup> Some samples of this compound tended to form oils. The oily material was, however, satisfactorily saponified and reduced.

ethyl nitrite in the presence of ethanol and hydrochloric acid.<sup>4</sup> The crude oximino ketone (V) was reduced directly to the amino ketone hydrochloride (VI) in the presence of palladium on charcoal catalyst and one equivalent of hydrochloric acid by the method developed by Hartung<sup>11</sup> and later applied to imidazole compounds by Ochiai and Ikuma.<sup>12,13</sup> The acidic solution of the crude amino ketone yielded ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazoleheptanoate (VII) upon treatment<sup>14</sup> with aqueous potassium cyanate. After saponification, the sodium salt of the imidazolone acid was hydrogenated at high pressure in the presence of Raney nickel catalyst by the procedure described by Wood and du Vigneaud<sup>6</sup> for the preparation of desthiobiotin. The yield of the optically inactive isomers from the dehydro compound was 75% of the theoretical amount.

Homodesthiobiotin, when tested by the yeast-growth method with *Saccharomyces cerevisiae*, Strain No. 139, was found to be completely devoid of biotin activity. Moreover, it inhibited the growth of yeast. This antagonism was overcome by adding more biotin to the medium.

The general procedure employed for the preparation of homodesthiobiotin from the keto ester was applied also to the preparation of desthiobiotin. Ethyl 8-oxononanoate,<sup>15</sup> prepared from ethyl  $\epsilon$ -bromocaproate<sup>16</sup> and acetoacetic ester and kindly supplied by Dr. George B. Brown of this Laboratory, was used as starting material. The desthiobiotin isomers which were obtained possessed 40% of the activity of biotin when tested by the yeast-growth procedure with *S. cerevisiae*.

When ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazolecarboxylate was saponified in an attempt to obtain the free acid for reduction to the corresponding imidazolidone, it was noted that large amounts of carbon dioxide were evolved upon acidification of the saponification mixture. Hy-

drogenation of the alkaline solution resulted in the formation of the previously undescribed 5-methyl-2-imidazolidone. These observations demonstrate the decarboxylation of the methyl-imidazolonecarboxylic acid during alkaline hydrolysis. In connection with this behavior, it is interesting to note that 2,3-dihydro-2-oxo-4-imidazolecarboxylic acid has been shown<sup>17</sup> to undergo decarboxylation when heated *in vacuo*. 5-Methyl-2-imidazolidone was found incapable of supporting the growth of yeast in place of biotin.

### Experimental<sup>18</sup>

**Ethyl 6-Oxoheptanoate.**— $\delta$ -Carbomethoxyvaleryl chloride (0.16 mole), b. p. 127° (15 mm.), was converted to ethyl 6-oxoheptanoate, b. p. 120-123° (13 mm.), by reaction in ether with dimethylcadmium prepared from magnesium (0.3 mole), excess methyl bromide and anhydrous cadmium chloride.<sup>9,10</sup> The yield of ketone was 59% of the theoretical amount. Cason and Prout<sup>10</sup> prepared this compound in a similar manner, but carried out the synthesis with benzene as the solvent to avoid production of contaminating di-ester which is formed when the ketone is prepared in ether solution. Our product prepared without this precaution was sufficiently pure, however, for the preparation of ethyl 2,3-dihydro-2-oxo-4-imidazolebutyrate.

**Diethyl Azelate.**—A solution of 122 g. of azelaic acid in absolute ethanol and sulfuric acid was heated under reflux for eighteen hours according to the method described for the preparation of diethyl sebacate.<sup>19</sup> The yield of di-ester, b. p. 144° (4 mm.), was 152 g. (96% of the theoretical amount). Jones and Smith<sup>20</sup> reported that the compound boiled at 130-132° (5 mm.). Chuit<sup>21</sup> reported a boiling point of 154-155° (8 mm.).

**Ethyl Hydrogen Azelate, II.**—The half ester, b. p. 173-178° (4 mm.), was prepared by the procedure described for the preparation of ethyl hydrogen sebacate.<sup>19</sup> The yield of mono-ester, m. p. 25-26°, was 58% of the theoretical amount.

**$\eta$ -Carbomethoxycaprylyl Chloride, III.**—A mixture of 45.0 g. of ethyl hydrogen azelate and 36 cc. of thionyl chloride was allowed to stand in an oil-bath at 50° for two hours and was finally heated under reflux at a bath temperature of 80° for two hours. Forty-five grams of the acid chloride, b. p. 158° (15 mm.), was obtained on distillation.

(11) Hartung, *THIS JOURNAL*, **53**, 2248 (1931).  
 (12) Ochiai and Ikuma, *J. Pharm. Soc. Japan*, **56**, 525 (1936); *C. A.*, **30**, 8212 (1936).  
 (13) Ochiai and Ikuma, *Ber.*, **69**, 1147 (1936).  
 (14) Gabriel and Posner, *ibid.*, **27**, 1141 (1894).  
 (15) Barger, Robinson and Smith, *J. Chem. Soc.*, 718 (1937).  
 (16) Brown and Partridge, *THIS JOURNAL*, **66**, 839 (1944).

(17) Hilbert, *ibid.*, **54**, 3413 (1932).  
 (18) All melting points were taken on a micro melting point apparatus.  
 (19) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, N. Y., 1943, p. 276.  
 (20) Jones and Smith, *J. Chem. Soc.*, 65 (1928).  
 (21) Chuit, *Helv. Chim. Acta*, **9**, 264 (1926).

The boiling point is in agreement with that reported by Ruzicka and Stoll.<sup>22</sup>

**Ethyl 9-Oxodecanoate, IV.**— $\eta$ -Carbethoxycaprylyl chloride (29.9 g.) was converted to the keto ester by reaction with dimethylcadmium in ether as noted under the preparation of ethyl 6-oxoheptanoate. The yield of keto ester, b. p. 145–148° (10 mm.), was 17.3 g. (64% of the theoretical amount). Barger, Robinson and Smith<sup>15</sup> reported that ethyl 9-oxodecanoate boils at 154–156° (13 mm.). Ruzicka and Stoll<sup>22</sup> reported a boiling point of 151–153° (11 mm.). Since the preparative procedure employed by us could have presumably resulted in contamination of the keto ester with di-ester,<sup>10</sup> our crude fraction was identified by conversion to the semicarbazone. The keto ester was found to be sufficiently pure for the preparation of homodesthiobiotin.

A semicarbazone was prepared from 170 mg. of the keto ester fraction by the general method for water-insoluble compounds.<sup>23</sup> The yield of glistening white product was 195 mg., m. p. 95–99°. A sample for analysis, m. p. 98–99°, was prepared by recrystallization from ethanol–water and then from 95% ethanol.

*Anal.* Calcd. for C<sub>13</sub>H<sub>25</sub>O<sub>3</sub>N<sub>3</sub>: N, 15.49. Found: N, 15.61.

Barger, Robinson and Smith<sup>15</sup> reported that the semicarbazone melted at 97–98°. Ruzicka and Stoll<sup>22</sup> reported a melting point of 102–103°.

**The  $\alpha$ -Oximino Ketones.**—The procedure employed for the preparation of these compounds was developed from the method previously reported.<sup>4</sup> A mixture of 0.04 mole of the keto ester, 4 cc. of 95% ethanol and 0.24 cc. of concentrated hydrochloric acid was warmed to 50° and then ethyl nitrite<sup>24</sup> was passed into the solution until 0.04 mole had been absorbed. The temperature of the reaction mixture was maintained at 50–55° throughout the course of the reaction by warming the mixture or, when necessary, by moderating the flow of gas so that the mixture did not become too hot. The mixture was then allowed to stand at room temperature for thirty to ninety minutes. The yellow-green to brown solution was then concentrated at the water pump at a temperature not exceeding 40°.<sup>25</sup> The crude material weighed within 10% of the calculated amount and was suitable for reduction without purification. The oximinoacetoacetic ester was prepared as described by Gabriel and Posner.<sup>14</sup>

**The  $\alpha$ -Amino Ketone Hydrochlorides.**—To a solution of 0.032 mole of the  $\alpha$ -oximino ketone in 32 cc. of 1 *N* hydrochloric acid and the minimum volume of 95% ethanol was added 0.5 g. of 5% palladium on charcoal catalyst. Reduction of the oxime was carried out at hydrogen pressures of 1–4 atmospheres until 60–100% of the calculated amount of hydrogen had been absorbed. The solution of the crude  $\alpha$ -amino ketone hydrochloride was separated from the catalyst and used directly for the preparation of the imidazolone ester.

**The Imidazolone Esters.**—The solution of the  $\alpha$ -amino ketone hydrochloride (0.032 mole) described above was cooled. A solution of 0.062 mole of potassium cyanate in a minimum quantity of water was added rapidly with stirring. The mixture was heated in the water-bath at 50° for about five minutes, allowed to stand at room temperature for one hour and finally cooled. The crystalline product usually separated during this time. If no separation occurred, the solution was diluted with water or evaporated to induce crystallization. The yield was 30–60% of the calculated amount. The esters were puri-

fied by recrystallization from ethanol or ethanol–water. Physical constants and analytical data for these compounds are reported in Table I.

**5-Methyl-2-oxo-4-imidazolidineheptanoic Acid (Homodesthiobiotin Isomers), VIII.**—To 10 cc. of 5% sodium carbonate solution was added 400 mg. of ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazoleheptanoate and 25 cc. of water. Saponification was effected by heating the mixture overnight at 100°. Concentrated hydrochloric acid was added to the resulting solution until it was just alkaline to phenolphthalein. About 1 g. of Raney nickel catalyst<sup>27</sup> was added. Hydrogenation was carried out at 100° and 150 atmospheres for forty hours. The catalyst was removed by filtration. The filtrate was made acidic to congo red by the addition of hydrochloric acid. The solution was then evaporated to a volume of about 40 cc. After the solution had been cooled for several hours at 5°, the white crystalline product was collected, and then washed with a small volume of cold water. The combined mother and wash liquors were then concentrated under reduced pressure to a volume of about 10 cc. An additional crop was obtained by cooling the concentrate at 5°. The yield of product, m. p. 120–135°, was 269 mg., 75% of the theoretical amount. The product was recrystallized once from water with no appreciable change in melting point.

*Anal.* Calcd. for C<sub>11</sub>H<sub>20</sub>O<sub>3</sub>N<sub>2</sub>: C, 57.87; H, 8.83. Found: C, 57.45; H, 8.65.

After one recrystallization from acetone followed by five recrystallizations from water the product melted at 140–150°. No change in biological potency resulted, however.

*Anal.* Calcd.: C, 57.87; H, 8.83. Found: C, 57.86; H, 8.90.

The change in melting range which resulted from the repeated recrystallizations may indicate a partial separation of isomers. This point requires further investigation.

**Desthiobiotin Isomers.**—Ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazolecaproate (1.20 g.) was hydrolyzed and reduced by the method described under the preparation of homodesthiobiotin. The reduction mixture was acidified and concentrated to a volume of about 20 cc. The solution was cooled at 10° overnight and deposited 401 mg. of white crystals, m. p. 140–155°. This material showed 40% of the activity of biotin on a weight basis when assayed by the yeast-growth method with *S. cerevisiae*. The mother liquors were again cooled and deposited an additional 236 mg. of white product which was not further investigated.

**5-Methyl-2-oxo-4-imidazolidineacetic Acid.**—To a suspension of 425 mg. of ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazoleacetate in 30 cc. of 5% sodium bicarbonate was added about 1 g. of Raney nickel catalyst. The mixture was hydrogenated at 100° and 150 atmospheres for forty hours. The mixture was then cooled to room temperature and separated from the catalyst by filtration. The filtrate was acidified to pH 5 by the addition of concentrated hydrochloric acid and then evaporated at the water pump to a volume of about 25 cc. The solution was extracted for twenty-four hours with ethyl acetate containing about 15% ether. The extract was concentrated to a residue (22 mg.) of oleaginous crystals, m. p. 140–144°. Then 2 cc. of 6 *N* hydrochloric acid was added to the aqueous solution, and it was extracted with ethyl acetate–ether for an additional forty-eight hours. The extract was concentrated to dryness at the water pump. The residue consisted of slightly colored crystals, m. p. 140–144°. This impure product was digested with 15 cc. of acetone. White prisms (11 mg.) were collected and washed with acetone. The material melted at 159–160°. The melting point was unchanged after several recrystallizations from ethanol and ethanol–water. An additional 55 mg. of compound was obtained from the acetone mother liquors.

(27) "Organic Syntheses," Vol. XXI, John Wiley and Sons Inc., New York, N. Y., 1941, p. 15.

(22) Ruzicka and Stoll, *Helv. Chim. Acta*, **10**, 691 (1927).

(23) Shriner and Fuson, "The Systematic Identification of Organic Compounds," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1940, p. 142.

(24) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 204.

(25) Marked discoloration was observed when higher temperatures were employed. Fox, Sargent and Buchanan<sup>26</sup> have recently reported the decomposition of an  $\alpha$ -oximino ketone on attempted distillation.

(26) Fox, Sargent and Buchanan, *This Journal*, **67**, 496 (1945).

*Anal.* Calcd. for  $C_8H_{10}O_3N_2$ : C, 45.56; H, 6.37; N, 17.71. Found: C, 45.24; H, 6.18; N, 17.88.

**Ethyl 2,3-Dihydro-5-methyl-2-oxo-4-imidazolecarboxylate.**— $\alpha$ -Oximinoacetoacetic ester was reduced to the amino ketone by the method described by Ochiai and Ikuma<sup>13</sup> except that 5% palladium on charcoal catalyst was used in place of the catalyst containing 40% metal described by them. Treatment of the crude amino ketone hydrochloride with potassium cyanate afforded 85% of the theoretical yield of imidazolone. In order to obtain this high yield it was necessary to acidify the original mother liquors to pH 4.5 with mineral acid. The micro melting point of the product was 220–221° which agrees with the melting point given by Gabriel and Posner.<sup>14</sup>

**5-Methyl-2-imidazolidone.**—A mixture of 2.55 g. of ethyl 2,3-dihydro-5-methyl-2-oxo-4-imidazolecarboxylate and 9 cc. of 2 *N* sodium hydroxide was heated at 90–95° for three hours. The resulting solution was then adjusted to pH 7 by the addition of hydrochloric acid (vigorous evolution of carbon dioxide) and 45 cc. of 5% sodium bicarbonate was added. The mixture was hydrogenated at 150 atmospheres and 100° for forty-five hours. The solution was filtered and then acidified to congo red by the addition of 50% sulfuric acid. The solution was then concentrated to a volume of 40 cc. under reduced pressure and finally extracted continuously with a mixture of ethyl acetate and ether (85:15 by volume) for several days. The extraction liquid was concentrated to dryness. The residual crude product, m. p. 110–116°, weighed 1.28 g., 86% of the theoretical amount. After one recrystallization from benzene the slightly colored crystals melted at 120–122°. A sample for analysis was obtained by sublimation at 75–80° (1 mm.). The compound then melted at 121–122°.

*Anal.* Calcd. for  $C_8H_9ON_2$ : C, 47.98; H, 8.05; N, 27.98. Found: C, 48.22; H, 7.94; N, 27.95.

It was also possible to obtain the cyclic urea in one step by carrying out the saponification, decarboxylation and reduction with excess sodium bicarbonate in the hydrogenation apparatus.

**Ethyl 5-Methyl-2-thiol-4-imidazoleacetate.**—To a solution containing 2.18 g. of ethyl  $\beta$ -aminolevulinic hydrochloride, prepared from ethyl levulinate<sup>15</sup> as described in the general procedures above, was added 2.1 g. of potassium thiocyanate in a minimum quantity of water. The mixture was heated at 65–70° in an open 50-cc. Erlenmeyer flask for fourteen hours. At the end of this period the volume of liquid was about 15 cc. White plates separated. The mixture was cooled to room temperature and the product (604 mg.) was collected and washed with a small quantity of water. The mother liquor deposited additional material when it was cooled to 5°, but this precipitate was not investigated. The thiolimidazole ester was recrystallized from 95% ethanol and then melted at 217–218°.

*Anal.* Calcd. for  $C_8H_{12}O_3N_2S$ : C, 47.97; H, 6.04; S, 16.01. Found: C, 47.73; H, 5.80; S, 16.46.

### Summary

The preparation of *homodesthiobiotin*, 5-methyl-2-oxo-4-imidazolidineheptanoic acid, from azelaic acid has been accomplished through the intermediate, ethyl 9-oxodecanoate. 5-Methyl-2-imidazolidone has been prepared and homologs in the dehydrodesthiobiotin series have also been described.

(28) Ruzicka, *Ber.*, **50**, 1362 (1917).

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## The Microbiological Synthesis of Riboflavin—A Theory Concerning its Inhibition

BY ABRAHAM LEVITON

In the microbiological synthesis of riboflavin, the presence of traces of iron salts results in striking decreases in yield. This phenomenon is of practical significance; and the definition of the lower limit of concentration at which a significant decrease in yield begins to take place forms the basis of one or more patents<sup>1</sup> which have been issued and put into practice.

For some time this Laboratory has been engaged in a study of the factors involved in the microbiological synthesis of riboflavin. In one series of experiments it was found that in media containing iron salts in critical concentrations, added riboflavin was destroyed. This result suggested that the action of iron was at least to some extent destructive rather than inhibitory. The explanation of the destructive action seemed to lie in the possibility that one or more riboflavin-destroying compounds were formed during the fermentation. The view that hydrogen peroxide in the presence of ferrous ion would bring about the decomposition of riboflavin suggested itself,

(1) Meade, Pollard and Rogers, U. S. Patent 2,239,680 (1942).

in spite of the fact that riboflavin in the presence of pure hydrogen peroxide is quite stable.

This paper is concerned with experiments which serve to support this view; and the results of both chemical and microbiological studies are presented. The chemical studies describing the influence of ferrous salts (ferric salts are ineffective) on the decomposition of riboflavin by hydrogen peroxide were designed to demonstrate the existence of a parallelism between the influence on one hand of ferrous ion concentration on the rate of decomposition of riboflavin by hydrogen peroxide and the influence on the other hand of ferrous ion concentration on the yield of microbiologically synthesized riboflavin. The chemical studies were designed furthermore to demonstrate the antagonistic effect of catalase and reducing agents on the action of hydrogen peroxide; and corresponding microbiological studies were designed to show that the effect of catalase and reducing agents on the yield of riboflavin and on the rate of fermentation could reasonably be ascribed to a mechanism involving hydrogen peroxide.