funnel. The flask was kept at or below room temperature while 0.55 mole of the acid chloride was added slowly with stirring. When the addition was complete, the mixture was allowed to come to room temperature. After standing for two to eighteen hours, it was heated to 80–100° for one-half to three hours. After cooling, the reaction mixture was washed repeatedly with water to remove any acid chloride, acid, inorganic salts, and, in the case of the lower alkyl lactates, any unreacted lactic ester. The product was then dried and distilled. Fresh cuprous chloride or, preferably, diamylhydroquinone was added before distillation, and carbon dioxide rather than air was supplied through an ebullition tube to prevent bumping during distillation. Yields and other data are recorded in Table I.

Reaction of Methacrylic Anhydride with Lactic Esters.—Commercial methacrylic anhydride was used. Redistillation before use was unnecessary.

The procedure was essentially the same as that in which the acid chlorides were used, except that the hydrogen chloride absorber was omitted and a few drops of sulfuric acid were added to catalyze the reaction. The reaction proceeded sluggishly and was incomplete, even when the mixtures were heated for five hours at 130–140°. Since methacrylic anhydride is not readily soluble in water and hydrolyzes slowly, it was not easily removed from the reaction mixture before distillation. When the lower alkyl lactates, especially methyl lactate, were used, the desired product and methacrylic anhydride had boiling points so close together that complete separation by distillation was difficult. Since methacrylic anhydride is a bifunctional polymerant, even traces of it in a monofunctional monomer profoundly alter the properties of the resin obtained by polymerization, a cross-linked polymer being obtained.

Polymerization Experiments.—The acrylates and methacrylates were polymerized in aqueous emulsion.

Fifty grams of monomer, 2 g. of Tergitol no. 4, 1 g. of Triton 720, and 100 g. of water were placed in a flask fitted with a paddle-type stirrer. The flask was placed in a bath kept at 100°, and ammonium persulfate was added, 10 mg. at a time, at intervals of thirty minutes, until polymerization began. Usually only one or two portions of catalyst were required. Heating and stirring were continued for three hours after polymerization began, although one hour appeared to be sufficient. The emulsions were steam-distilled to remove monomer or volatile impurities. Brine was added to break the emulsions, and the polymers were then washed on a small washing mill. Yields were virtually quantitative.

Since some of the polymers were too soft and tacky to be molded and handled in sheet form, and hence could not be readily tested on the standard brittle-point apparatus, the brittle points of all the polymers were determined by the rather crude procedure of immersing a strip in a cooled ethanol bath, grasping it with tongs, and flexing. A water-bath was used for determinations above room temperature. Results could be duplicated within about ±3° by this method. The brittle points of the polymers are included in Table I.

Some of the monomers of Table I were polymerized in mass and in ethyl acetate solution. The products obtained by mass polymerization were transparent and colorless; the solutions of the polymers were colorless and clear.

Determination of Cross-Linking Tendency.—The method[12] described previously was used.

Summary

α-Carbalkoxyethyl acrylates and methacrylates were prepared by acylating methyl, ethyl, isopropyl, *n*-butyl, isobutyl, cyclohexyl, allyl, methallyl, and methylvinylcarbinyl lactates with acrylyl chloride, methacrylyl chloride or methacrylic anhydride. Polymerization of these unsaturated esters yielded colorless and transparent resins. The esters having two olefinic linkages yielded insoluble and infusible polymers. The cross-linking tendency of the bifunctional monomers was less than that of methallyl acrylate but greater than that of citronellyl, furfuryl, or crotyl acrylate.

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

The Synthesis of Optically Inactive Desthiobiotin

BY JOHN L. WOOD AND VINCENT DU VIGNEAUD[1]

Desthiobiotin, ε-(4-methyl-5-imidazolidone-2)-caproic acid, has been found to be as effective as an equal weight of biotin in supporting the growth of yeast. It also has been shown to exhibit antibiotin activity toward some microörganisms.[2,3] The optically active compound ($[\alpha]^{21}D$ +10.7°) has been obtained by degradation of biotin by the action of Raney nickel on the methyl ester[4] or the sodium salt.[5] It has furthermore been resynthesized from its further degradation product, ζ,η-diaminopelargonic acid, by the action of phosgene in alkaline solution.[6]

A method of total synthesis of *dl*-desthiobiotin which promises to be a practical means of obtaining the compound in quantity has been devised.

α-Aminosuberic acid, III, was prepared by the malonic ester method from ethyl ε-bromocaproate, I. The amino acid was treated with acetic anhydride and pyridine according to the method of Dakin and West.[7] The resulting ζ-keto, η-acetoaminopelargonic acid, IV, was hydrolyzed to the amino ketone derivative, V, and this was treated with potassium cyanate to produce ε-(4-methyl-5-imidazolone-2)-caproic acid, VI. The sodium salt of the imidazolone was reduced with hydrogen and Raney nickel catalyst at 150 atmospheres

(1) The authors wish to express their appreciation to Mr. Roscoe Funk of this Laboratory for carrying out the microanalyses and to Dr. Karl Dittmer, Mrs. Glenn Ellis and Miss Kate Redmond for the microbiological assays.

(2) Dittmer, Melville and du Vigneaud, *Science*, **99**, 203 (1944).

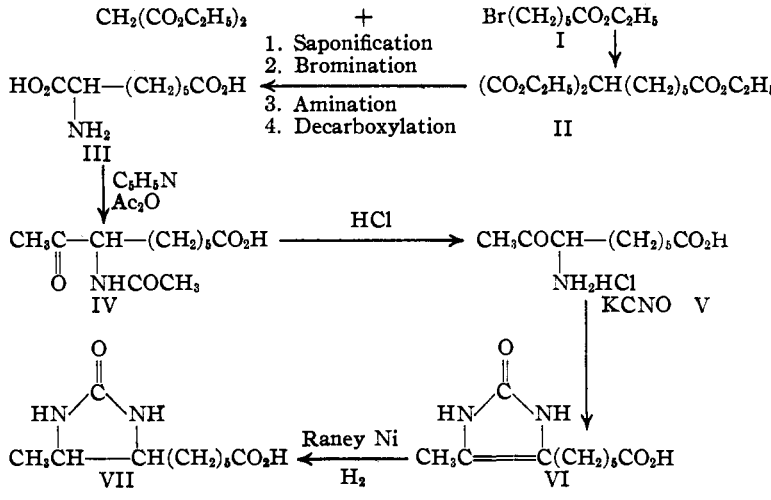
(3) Lilly and Leonian, *ibid.*, **99**, 205 (1944).

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

(5) Melville, Dittmer, Brown and du Vigneaud, *Science*, **98**, 497 (1943).

(6) Melville, THIS JOURNAL, **66**, 1422 (1944).

(7) Dakin and West, *J. Biol. Chem.*, **78**, 91, 745, 757 (1928).



and 100°. The reduction product, VII, consisted of *dl*-desthiobiotin and *dl*-allodesthiobiotin which differ from one another only in the configurational relationships of the carbon atoms 4 and 5 of the imidazolidone ring.

For proof of the structure of the products, the mixture was hydrolyzed to ζ,η-diaminopelargonic acid under the same conditions that were applied to desthiobiotin obtained from biotin. The dibenzoquinoxaline derivative was prepared by the reaction of the diaminopelargonic acid with phenanthrenequinone. This derivative was shown to be identical with the corresponding dibenzoquinoxaline derivative prepared from the degradation products of biotin.[4] The identity of the chemical structure of the synthetic product with "natural" desthiobiotin was thus established.

Experiments on the separation of the diastereoisomers were only in the preliminary stages when this work was interrupted by the war program. In one experiment, a sample having 55–60% of the microbiological activity of "natural" desthiobiotin was obtained by fractional elution of the substances adsorbed on Permutit. Because of the continued interest in the biological properties of desthiobiotin,[2,3,8] the synthesis is reported at its present stage of development. Without any separation, the mixture of isomers showed 30–35% of the activity of biotin when assayed with *Saccharomyces cerevisiae*, Strain No. 139. It was thus effective in producing a half-maximum growth increase at a concentration of 1 part in 1.5×10^{10}.

Experimental

Diethyl α-Carbethoxysuberate, II.—Ethyl ε-bromocaproate, I, was prepared according to the method of Brown and Partridge.[9] A solution of 240 g. of redistilled ethyl malonate and 17.1 g. of sodium in 450 cc. of absolute ethanol was prepared in a 3-liter 3-necked flask equipped with mercury-sealed stirrer, reflux condenser and dropping funnel. A small portion of 162 g. of the ethyl ε-bromocaproate was added with stirring and warming until separation of sodium bromide indicated the start of the reaction. The rest of the ester was added at a rate to keep the mixture refluxing. Heating at reflux temperature and stirring was continued for twenty-four hours. The condenser was set for downward distillation and the alcohol was distilled out of the mixture. The residue was poured into ice water. The organic layer was shaken with saturated sodium chloride solution twice and then was dried over sodium sulfate. The material was distilled from a Claisen flask in a boiling water-bath to collect a forerun of malonic ester. The desired product was collected from 140° (uncor.) at 1–2 mm. The temperature of the distillate was 167–170° (uncor.) for the most part. The yield was 174 g. or 76% of the theoretical.

α-Aminosuberic Acid, III.—A mixture of 168 g. of the diethyl α-carbethoxysuberate, 100 g. of potassium hydroxide, 40 cc. of water, and 400 cc. of 95% alcohol was heated to boiling under reflux for three hours. To this were added 50 g. of potassium hydroxide and 200 cc. of 50% alcohol. The mixture was stirred and heated for two hours. The alcohol was distilled with stirring; the last traces were removed *in vacuo*. The residue was cooled to 0–10° with an ice–salt bath. Then 900 cc. of alcohol-free ether and 250 cc. of concentrated hydrochloric acid were added dropwise. The potassium chloride precipitate was dissolved in added water. The aqueous layer was extracted by three 100-cc. portions of ether. The combined ether extracts were washed with 100 cc. of saturated sodium chloride and filtered through a filter paper containing anhydrous sodium sulfate. Bromine was added and the solution was illuminated with a 500-watt electric bulb to induce decolorization. When the reaction had started, decolorization of added bromine continued rapidly in diffuse daylight. After 33 cc. of bromine had been added, reaction ceased. The hydrobromic acid was washed out of the ether with two portions of water. The ether layer was poured into 1500 cc. of cooled concentrated ammonium hydroxide and this was allowed to stand forty-eight hours.

The resultant solution was distilled under reduced pressure until acid to litmus. Five hundred cc. of water and 200 cc. of concentrated hydrochloric acid were added. The solution was heated in a boiling water-bath for two and one-half hours. It was concentrated until a solid cake was obtained. Concentrated ammonium hydroxide was added until the solution was alkaline to congo red and acid to litmus. After it had stood in the refrigerator overnight, the precipitated acid was filtered. The product was dissolved in hot dilute ammonium hydroxide. The solution was filtered and neutralized. The precipitated material was washed on the funnel with water and then with alcohol until halogen free. After drying *in vacuo* at 70°, the product weighed 63 g. The mother liquor was concentrated and yielded 6.5 g. This represents a yield of 57% of the theoretical amount.

A sample of α-aminosuberic acid prepared as above was recrystallized from hot water. It melted at 231–233°[10] with decomposition when placed in the bath at 227°.

Anal. Calcd. for $C_8H_{15}O_4N$: N, 7.36. Found: N, 7.27.

A sample of the malonic acid was isolated from a trial run of the saponification to check on the identity of the product. It was a crystalline solid of m. p. 95–98°..

Anal. Calcd. for $C_8H_{14}O_6$: C, 49.53; H, 6.46. Found: C, 49.25; H, 6.15.

ε-(4-Methyl-5-imidazolone-2)-caproic Acid, VI.—A suspension of 7.6 g. of α-aminosuberic acid in 50 cc. of acetic anhydride and 40 cc. of pyridine was heated in a water-bath at 80–90° for four hours. The solvent was distilled

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

(9) Brown and Partridge, THIS JOURNAL, **66**, 839 (1944).

(10) All melting points are corrected.

in vacuo at 50°. Water was added to the residue and then distilled out at reduced pressure. The process was repeated. Sixty cc. of 2 *N* hydrochloric acid was added and the solution was heated under reflux for four hours. The resultant solution was treated with Norite and then concentrated to dryness under nitrogen *in vacuo* at 40°. The residue was taken up in 20 cc. of water. About 3–5 g. of solid sodium acetate was added and followed by saturated sodium carbonate until the *p*H was 4.5–5. The solution was cooled in an ice-bath and 5 g. of potassium cyanate in 15 cc. of water was added. The *p*H was adjusted to 6–6.5 with 5 *N* sodium hydroxide. It was warmed to 40° and allowed to stand one-half to one hour at room temperature. The solution was then adjusted to *p*H 4 by the addition of dilute hydrochloric acid and cooled in an ice-bath. The product separated as a thick mass which was filtered and washed with ice water. The pressed precipitate was dissolved immediately in a minimum of hot absolute alcohol and poured into about 10 volumes of water. After cooling in the refrigerator, it was filtered and dried *in vacuo* over phosphorus pentoxide at room temperature.

The product was hygroscopic and usually had some color (weight, 4.2–4.8 g., 50–56%). When placed in a bath at 158°, it melted at 168° with decomposition.

Anal. Calcd. for $C_{10}H_{16}O_3N_2$: N, 13.20. Found: N, 12.85.

The product gave a characteristic purple-colored solid when treated with bromine in carbon tetrachloride.

Desthiobiotin Isomers, VII.—A 5% sodium bicarbonate solution was added to 10 g. of the imidazolone until a *p*H of 7.5 was attained. The solution was filtered and diluted to 120 cc. with water. Approximately 10 cc. of Raney Ni catalyst was added. The mixture was placed in a 300-cc. bomb and shaken at 2300 lb. pressure and 100° for thirty-six hours. The solution was filtered from the catalyst and the *p*H (11.3) was reduced to 8.5 with 10 *N* sulfuric acid. This was decolorized with Norite and then acidified to Congo red with 10 *N* sulfuric acid. The product separated as colorless crystals. These were washed with ice water and dried in a desiccator over phosphorus pentoxide (weight, 6.3 g., m. p. 136–137°). On cooling, the mother liquor deposited 1.2 g. of rosets, m. p. 135–139°. On concentration of the mother liquor a further crop of 0.78 g., m. p. 130–136°, was isolated. The analyses indicated desthiobiotin had been formed by the reduction.

Anal. Calcd. for $C_{10}H_{18}O_3N_2$: C, 56.03; H, 8.46; N, 13.07. Found: C, 56.14; H, 8.37; N, 12.99, 12.84, 13.22.

By assay with yeast, the first crop of crystals was found to have 30–35% of the activity of an equal weight of biotin. The second was assayed at 20–25%.

As a further check the structure of the product was established by conversion to diaminopelargonic acid by the action of barium hydroxide.[4] The diamino acid was converted to the dibenzoquinoxaline derivative by the action of phenanthrenequinone. This derivative was shown to be identical with the derivative obtained from desthiobiotin prepared from biotin by comparison of its physical properties. A mixture of the two dibenzoquinoxaline samples showed no depression of the melting point.

Due to the presence of the two asymmetric carbons, the synthetic desthiobiotin might be expected to consist of a mixture of the two racemic forms. A sample of the desthiobiotin isomers showed little change in microbiological activity or in melting point with repeated crystallization from water. Some indication of fractionation was obtained. One hundred milligrams of the mixture was dissolved in 10 cc. of water and passed over 10 cc. of Permutit. The effluent solution was concentrated to obtain 31 mg. of product melting at 151–154°. After recrystallization from water, 25 mg. was obtained which melted at 157–159° and showed 55–60% of the activity of an equal weight of biotin on yeast. The mother liquors yielded 41 mg. of crystalline material, m. p. 138–140°, assaying at 35–40% of biotin.

Summary

A total synthesis of *dl*-desthiobiotin has been reported. The product obtained appeared to be a mixture of the two possible racemic forms. This mixture is one-third as active as biotin in promoting the growth of yeast in a medium deficient in biotin.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Study of the Essential Groups of β-Amylase.[1] I

BY C. EDWIN WEILL[2] AND M. L. CALDWELL

Selective reagents which react with free groups of proteins have become increasingly important in protein chemistry. They are of special value in the study of proteins which have characteristic activities such as certain hormones and enzymes. By careful choice of such reagents, and of the conditions, it is often possible to block or to alter one or more of the free groups of a protein without affecting the others, and, when there is characteristic activity, to ascertain which, if any, of these groups is connected with or essential to its activity. The groups which have been studied most often are the free amino,[3] the free tyrosine[4] and the free sulfhydryl groups[5] of proteins.

The present investigation deals with a study of the influence of a number of selective reagents upon the activity of β-amylase from barley and

(1) Grateful acknowledgment is made to Mr. Robert Schwarz of the Schwarz Laboratories, Inc., and to The Ladish-Stoppenbach Company, who kindly furnished the barley and malted barley used in this investigation.

(2) This work is taken from a dissertation submitted by C. Edwin Weill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University. The major portion of this paper was presented at the New York meeting of the American Chemical Society, September, 1944.

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