times with ethyl acetate. The remaining aqueous portion then was filtered to remove the brown humin and diluted to a known volume with 1 *N* HCl. The optical density of the aqueous portion was determined at 390 mμ and the amount of ε-DNP-lysine calculated by comparison with a standard curve.[35] In some cases, the method of Porter[36] and that of Levy[15] were used.

The ether–ethyl acetate solution was evaporated to dryness in a current of warm air. The residue was taken up in a small amount of water-saturated chloroform. This solution then was placed on a silica gel column as described by Porter[36] and the DNP-valine separated from dinitrophenol, dinitroaniline and DNP-peptides. After separation was complete, the column was extruded, and the DNP-derivatives eluted with acetone, evaporated to dryness, dissolved in 1% $NaHCO_3$ solution and the optical density determined at 350 mμ. The moles of DNP-residue were determined using 17,000 as the molar extinction coefficient for DNP-valine. The extinction coefficient for the DNP-peptide was also assumed to be 17,000.[34]

The results of recovery experiments of DL-valine when hydrolyzed in the presence of DNP-chymotrypsin and DNP-lysozyme are recorded in Table III. Approximately 1 mg. of DNP-valine and 20 mg. of DNP-protein together with 1 ml. of constant boiling HCl were hydrolyzed for the periods of time indicated. The results represent the average of at least 4, and in some cases 6, determinations, as do the results of Tables II.

TABLE III

DNP-DL-VALINE RECOVERIES

Hydrolysis time (hr.)	% Recovery DNP-chymotrypsin	% Recovery DNP-lysozyme
16	82	80
27	78	75
35	72	69
72	65	60
96	61	..

The results reported here show slightly less destruction of DNP-valine than observed by Porter and Sanger[37] and Masri and Singer.[38] However, the losses are somewhat higher than the 13% reported recently by Rhinesmith, Schroeder and Pauling[12] for a 22-hour hydrolysis in the presence or absence of DNP-globin.

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Elaiomycin. II. Determination of the D-*threo* Configuration

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The tuberculostatic antibiotic Elaiomycin (I) has previously been shown to contain two asymmetric carbon atoms, both situated in a C_5-moiety, which has been degraded to a derivative II of 3-amino-4-methoxy-2-butanol. Elaiomycin has been shown by independent synthesis of an optically active derivative II to have the D-*threo* configuration.

In the previous paper in this series[2] the structure of Elaiomycin was proved to be I by degradation experiments. Complete hydrogenation of I resulted in hydrogenolysis of the N-to-N bonds with the formation of octylamine and a methoxyamino alcohol. The structure of this amino alcohol was confirmed by the synthesis of a diastereoisomeric mixture of II.

In this work the amino alcohol was first shown to be in the *threo* series by synthesis of DL-II starting from DL-threonine. The ethyl ester hydrochloride of DL-threonine was treated with methyl iminobenzoate by the method of Elliott[3] to give *threo*-DL-5-methyl-2-phenyl-2-oxazoline-4-carboxylic acid ethyl ester (III), which has been shown to be formed with retention of the *threo* configuration.

The reduction of III with excess lithium aluminum hydride resulted in the cleavage of the oxazoline ring and formation of N-benzyl-DL-threoninol (IV) in 87.5% yield. By careful reduction of III with an equivalent amount of lithium aluminum hydride *threo*-DL-5-methyl-2-phenyl-2-oxazoline-4-methanol (V) was obtained in 88% yield. Lithium aluminum hydride has previously been shown to reduce esters of optically active aminoacids to the corresponding alcohols without racemization.[4]

Tosylation of V in pyridine at 0° gave the *p*-toluenesulfonate of V in 75–99% yields. Subsequent treatment of the tosylate VI for 84 hours with sodium methoxide in absolute methanol resulted in the formation of the *threo*-DL-5-methyl-2-phenyl-4-methoxymethyl-2-oxazoline(VII) in good yield. Hydrolysis of the oxazoline ring in VII, and acetylation of the resulting amino alcohol gave the desired DL-*threo*-3-acetamido-4-methoxy-2-butanol acetate (II), m.p. 78.2–79°. The infrared absorption spectra of a solution of II and of the acetylated amino alcohol from Elaiomycin were superimposable. A mixture melting point determination of the two was depressed to 70°. The identity of the spectra of the two compounds definitely placed the degradation product from Elaiomycin in the *threo* series.

The utilization of L-threonine and the procedures developed for synthesis of the DL-*threo* compounds resulted in the isolation of L-*threo*-3-acetamido-4-methoxy-2-butanol acetate, which derivative had the same melting point, 76.4–76.8°, as that from Elaiomycin but opposite rotation. These data and the fact that the infrared spectra of the two compounds were identical prove that the natural

(1) This work was supported in part by a Parke-Davis Research Fellowship.

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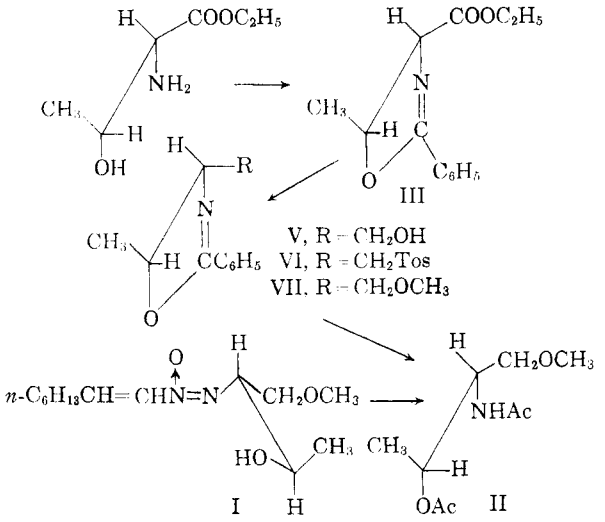

amino alcohol derivative and consequently Elaiomycin exists in the D-*threo* configuration.

The fact that DL-II, produced by synthesis from DL-threonine, melted higher than D- or L-II indicated racemic compound formation, which was confirmed by mixture melting point studies. A 50–50 mixture of L-II (synthetic) with D-II (natural) raised the melting point of each to 79–80° and the melt, when cooled and mixed with DL-II (from DL-threonine), did not change the melting point of the latter compound. A mixture of composition 25% L and 75% D or 75% L and 25% D melted at 70°.

With the exception of the final derivative II, and the L-*threo*-tosylate which was not isolated as a solid, the intermediate solid compounds of the L-*threo* configuration melted higher than their corresponding DL-*threo* isomers.

Experimental

threo-DL-**5-Methyl-2-phenyl-2-oxazoline-4-carboxylic Acid Ethyl Ester (III).**—DL-Threonine was esterified and the ethyl DL-threoninate hydrochloride was treated with methyl benzimidate according to the procedure of Elliott.[3] From 6.08 g. of ethyl DL-threoninate hydrochloride was obtained 6.12 g. (80%) of DL-*threo*-2-phenyl-5-methyl-4-carbethoxy-2-oxazoline, b.p. 126° (0.5 mm.), $n^{25}D$ 1.5255.

N-Benzyl-DL-threoninol (IV).—To 1.85 g. (0.0486 mole) of lithium aluminum hydride in ether at 0° was added with stirring 9.46 g. (0.0405 mole) of III. After two hours at reflux, the excess lithium aluminum hydride was decomposed with moist ether and a little water. The reaction mixture was filtered and the aluminum hydroxide and lithium salts were extracted three times with absolute ethanol. Ethanol was removed, ether was added and after filtration the filtrate was combined with the original ether solution. Removal of the ether and distillation gave 6.58 g. (84.3%) of N-benzyl-DL-threoninol, b.p. 132–135° (0.4 mm.), $n^{25}D$ 1.5367.

Anal. Calcd. for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.77. Found: C, 67.58; H, 8.67.

threo-DL-**5-Methyl-2-phenyl-2-oxazoline-4-methanol (V).**—To 1.14 g. (0.0302 mole) of lithium aluminum hydride in ether at 0° was added rapidly and with stirring 12.3 g. (0.0527 mole) of III. The reaction was allowed to come to room temperature and then stirred 1.5 hours. Moist ether and a little water was added to decompose any excess hydride. The ether layer was separated and the aqueous layer was extracted three times with 75-ml. portions of ether. The ether layers were combined and dried over anhydrous sodium sulfate. The ether was removed after filtration to leave 8.8 g. (88%) of V as white crystals, m.p. 81–83°. A small sample was recrystallized from ether for analysis, m.p. 85–86°, λ_{max} 240 mμ, ε 10,700, in 95% ethanol.

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 69.08; H, 6.85. Found: C, 69.05; H, 7.15.

threo-DL-**5-Methyl-2-phenyl-2-oxazoline-4-methanol *p*-Toluenesulfonate (VI).**—A solution of 4.1 g. (0.0214 mole) of V in anhydrous pyridine was cooled to 0° and added to 4.19 g. (0.022 mole) of *p*-toluenesulfonyl chloride in pyridine and the reaction mixture allowed to stand at 0° for 8 hours. Pyridine was then removed under vacuum and anhydrous benzene added, after which the reaction mixture was filtered to remove the pyridine hydrochloride. Ether and more benzene were added which caused the precipitation of an insoluble oil. Removal of solvent under reduced pressure caused crystallization and left 5.55 g. (75%) of the *p*-toluenesulfonate of V as a white crystalline solid, m.p. 75–76°. A previous run yielded 100% of somewhat less pure product, m.p. 71–73°.

Anal. Calcd. for $C_{18}H_{19}NO_4S$: C, 62.58; H, 5.54. Found: C, 62.91; H, 5.73.

threo-DL-**5-Methyl-2-phenyl-4-methoxymethyl-2-oxazoline (VII).**—Treatment of 7.5 g. (0.0217 mole) of the above tosylate with 2.16 g. (0.04 mole) of sodium methoxide in excess anhydrous methanol at reflux temperature for 84 hours followed by removal of methanol under reduced pressure left a solid and an oil as the residue. This residue was taken up in ether and water and the layers separated. The water layer was extracted with ether and the combined ether solutions were dried over sodium sulfate. After filtration and removal of the ether, the resulting liquid was distilled to give 3.34 g. (78%) of VII, b.p. 90–92.5° (0.5 mm.), $n^{25}D$ 1.5300, λ_{max} 240 mμ, ε 10,900, in 95% ethanol.

Anal. Calcd. for $C_{12}H_{15}NO_2$: C, 70.21; H, 7.36. Found: C, 70.50; H, 7.43.

threo-DL-**3-Acetamido-4-methoxy-2-butanol Acetate (II).**—Hydrolysis of 1.63 g. (0.0079 mole) of VII with 20 ml. of 6 *N* hydrochloric acid for 5 hours at the reflux temperature followed by cooling resulted in the crystallization of benzoic acid. Extraction with three 25-ml. portions of ether and evaporation of the ether layers left crude benzoic acid which was recrystallized from ethanol–water to yield 0.55 g., m.p. 120–121°. The aqueous layer from the extraction was evaporated to leave a white amine hydrochloride, 1.15 g. (93% crude). Treatment of the amine hydrochloride with excess sodium methoxide in a minimum amount of methanol liberated the amine. A large excess of acetic anhydride was slowly added and permitted to react with the solution of the amine. After heating the acetylation mixture for 2 hours, the mixture was filtered. The volatile components were removed under reduced pressure and the residue was treated with 40 ml. of anhydrous ether and then filtered. The filtrate was reduced in volume and transferred to a 2-ml. distillation flask. Distillation afforded a liquid, b.p. 112–115° (0.4 mm.), $n^{25}D$ 1.4576, which crystallized to yield 0.65 g. (45%) of solid *threo*-DL-3-acetamido-4-methoxy-2-butanol acetate, m.p. 78.2–79°. An infrared spectrum of this compound in carbon tetrachloride was superimposable with completely acetylated II (from Elaiomycin) in the same solvent.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.18; H, 8.43; N, 6.89. Found: C, 53.54; H, 8.62; N, 7.19.

threo-L-**5-Methyl-2-phenyl-2-oxazoline-4-carboxylic Acid Ethyl Ester (L-III).**—Utilization of the same procedure as for DL-III and employing 11.7 g. of ethyl L-threoninate hydrochloride gave 6.49 g. of the L-isomer, b.p. 132–138° (1 mm.), $n^{25}D$ 1.5262, $[\alpha]^{25}D$ +89.7° (5.4% in $CHCl_3$). The infrared spectra of this compound in solution was identical with its corresponding DL-isomer.

threo-L-**5-Methyl-2-phenyl-2-oxazoline-4-methanol (L-V).**—The same procedure was used as in the preparation of V. From 14 g. (0.06 mole) of L-III and 1.37 g. (0.036 mole) of lithium aluminum anhydride was obtained 8.2 g. (72%) of the L-methanol, m.p. 96–97.5°. A small sample was recrystallized from ether for analysis and physical constants, m.p. 99.5–100°, $[\alpha]^{25}D$ +79.2° (1.63% in $CHCl_3$).

Anal. Calcd. for $C_{11}H_{13}NO_2$: C, 69.08; H, 6.85. Found: C, 69.14; H, 6.93.

threo-L-**5-Methyl-2-phenyl-4-methoxymethyl-2-oxazoline (L-VII).**—To 6.86 g. (0.036 mole) of *p*-toluenesulfonyl chloride in pyridine at 0° was added 6.5 g. (0.034 mole) of the L-methanol derivative dissolved in pyridine. The reaction mixture was stored for 8 hours at 15°. The pyridine

was then removed under reduced pressure. Benzene and ether were added and the solution was decanted from the pyridine hydrochloride. The benzene and ether were evaporated to leave a viscous residue. This residue was taken up in a hexane–ether mixture and filtered from further precipitated salts. Removal of the solvents under vacuum left 9.2 g. (78%) of the semi-solid tosylate which was used directly in the following reaction. Treatment of the 9.2 g. (0.0266 mole) of the tosylate with 2.26 g. (0.042 mole) of sodium methoxide in excess methanol for 84 hours at reflux and utilization of the procedure developed for the synthesis of DL-VII resulted in the formation of 3.88 g. (71%) of L-VII, b.p. 87–89° (0.4 mm.), $n^{25}D$ 1.5292, $[\alpha]^{25}D$ +56.4° (1.81% in $CHCl_3$).

Anal. Calcd. for $C_{12}H_{15}NO_2$: C, 70.21; H, 7.36. Found: C, 70.49; H, 7.48.

L-*threo*-3-Acetamido-4-methoxy-2-butanol Acetate (L-II).—The *threo*-L-5-methyl-2-phenyl-4-methoxymethyl-2-oxazoline (3.075 g., 0.015 mole) was treated with 40 ml. of 6 *N* hydrochloric acid at the reflux temperature for 6 hours, after which the product was separated from benzoic acid and acetylated as described for DL-II. Distillation gave 1.3 g. (43%) of L-II, b.p. 105–107° (0.3 mm.), m.p. 76.4–76.8°, $[\alpha]^{25}D$ +7.2° (1% solution in chloroform). The infrared spectrum of this compound in solution was identical and superimposable with the spectra of the DL-*threo*-isomer and with the D-*threo*-isomer obtained from Elaiomycin. A mixture melting point determination with the DL-*threo*-isomer VII melted at 70° while a determination with an equal amount of II from Elaiomycin raised the melting point to 79.8–80.2° indicating compound formation.

DETROIT 2, MICH.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Transglucosylation by a Mammalian Liver Enzyme

BY MARJORIE R. STETTEN

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The mechanism of action of a rat liver enzyme, termed transglucosylase, capable of transferring glucosyl residues from maltose, maltotriose or glycogen to glucose, maltose and higher homologs or to water has been studied. Glucose-C^{14} is introduced by the enzyme into only the reducing terminal sugar of the oligosaccharides. It was shown that, whereas the transfer of glucosyl residues from one sugar to another is reversible, the delivery of glucosyl residues to water is irreversible. The enzymatic reaction is a slow one and does not require the participation of phosphate or other dialyzable co-factors.

The occurrence of transglycosylation reactions, resulting in the formation of oligosaccharides or polysaccharides by enzyme preparations from various plants, bacteria and molds has been shown to be widespread and has been extensively studied and well reviewed.[1,2] That such intermolecular transfer reactions may occur also in animal tissues is indicated by the progressive appearance of a series of oligosaccharides on incubation of rat liver homogenates with maltose[3] and the finding of significant amounts of such oligosaccharides in rat diaphragm[4] and normal liver.[5]

The mechanism generally proposed for such reactions involves the transfer of a glucosyl moiety from the non-reducing end of an oligosaccharide to the enzyme with the formation of a glucosyl enzyme complex. The glucosyl group is then pictured as being transferred either to a suitable carbohydrate acceptor or to water. Most, but not all[2] of the experimental observations with bacterial and plant enzyme preparations have been consistent with this concept. Studies on the nature and reversibility of the transglucosylation reaction

$$2 \text{ maltose} \rightleftharpoons \text{glucose} + \text{maltotriose} \qquad (1)$$

carried out by enzyme preparations from rat liver are here reported.

An alcohol precipitated powder obtained from the supernatant solution of rat liver homogenates was used as the source of enzyme activity. When an extract of the enzyme preparation in sodium acetate buffer of *p*H 5.0 was incubated at 37° with added maltose, liberation of glucose was observed, accompanied by the slower progressive accumulation of maltotriose and higher oligosaccharides in the medium. The synthetic reaction, with the accumulation of oligosaccharides, could be favored by the addition of glucose oxidase to the reaction mixture containing maltose and a dialyzed clear supernatant extract as the enzyme (Fig. 1). In the presence of boiled enzyme no reaction occurred. Glucose-C^{14}, introduced into the active reaction mixture, was incorporated into the maltose and oligosaccharides. Maltose-C^{14} and maltotriose-C^{14} so prepared were isolated and purified chromatographically (Fig. 2). Maltose-C^{14} was characterized by conversion to its octaacetate, dilution with authentic maltose octaacetate and recrystalli-

TABLE I

RATE OF APPEARANCE OF GLUCOSE FROM A CRUDE TRANSGLUCOSYLASE PREPARATION WITH AND WITHOUT ADDED MALTOSE

Time, hr.	Active enzyme[b] No added substrate, γ glucose/ml.	Active enzyme[b] 1000 γ maltose/ml., γ glucose/ml.[a]	Boiled enzyme 1000 γ maltose/ml., γ glucose/ml.[a]
0	4.8	4.2	4.8
1	5.0	127	6.9
2	5.2	150	5.2
4	3.6	287	7.0
6	..	326	..
12	7.8	449	6.3
24	17.3	755	8.5
Total on acid hydrolysis	64[c]	1074	

[a] Values corrected for 8 γ of glucose/ml. produced by glucose oxidase (Worthington Biochemical Corp.) under the same conditions from 1000 γ maltose/ml. [b] Five mg. enzyme powder per ml. of acetate buffer, *p*H 5.0. [c] In other experiments, when only the soluble portion of the dialyzed enzyme was used, there was no glucose precursor in the enzyme solution.

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