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Furan and Tetrahydrofuran Derivatives. VII. The Synthesis and Biological Activity of a Number of Oxybiotin Homologs[1]

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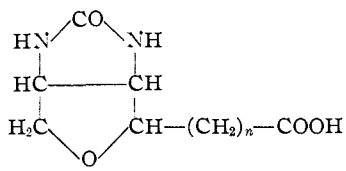
In recent communications[2,3,4] suitable procedures have been described for the preparation of hexahydro - 2 - oxo - 1 - furo(3,4)imidazoles. These methods later were utilized in the total synthesis of *dl*-oxybiotin,[5] the oxygen analog of biotin. The availability of practical methods for the preparation of this class of compounds made possible a systematic study of the relationships of chemical structure and biological activity in the oxybiotin series. Such investigations, it is hoped, may offer clues as to the active centers within the oxybiotin molecule and thus be useful in clarifying the mode of action of this substance. Similar studies in the biotin series are complicated by the difficulties involved in obtaining the necessary compounds. However, relevant information may be obtained from studies of oxybiotin derivatives, since it is to be expected that the biological activity of such compounds would parallel that of the corresponding biotin derivatives.

The observation that the replacement of the sulfur by oxygen does not significantly alter the biological activity of biotin,[6,7] coupled with the finding that oxybiotin has intrinsic biotin-like activity[8,9] has already demonstrated the relative non-specificity of the tetrahydrothiophene moiety of the molecule.

A systematic study of the biological activity of oxybiotin homologs was undertaken to determine the specificity of the valeric acid side chain. A series of compounds of the general structure (I) were prepared in which n was varied from 2 to 6, and were tested for their biological activity. In



(I)

view of the fact that the oxybiotin molecule contains three asymmetric carbon atoms and therefore may exist in several stereoisomeric forms, it was necessary to correlate all of the above homologs with oxybiotin by direct chemical procedures. Only a comparison of homologs with identical stereochemical configurations can yield the desired information on the relation of side-chain length to biological activity.

Starting material for the preparation of the lower homologs of this series, namely, *dl*-bisnor-oxybiotin (n = 2) and *dl*-nor-oxybiotin (n = 3) was 2-furanpropanol (II). This substance was originally prepared by catalytic hydrogenation of furylacrolein over an activated platinum catalyst.[10] We have prepared 2-furanpropanol by reduction of furylacrolein over a Raney nickel catalyst, and have found this procedure more practical than the original preparation.

2-Furanpropanol (II) was subjected to the Alder–Rickert procedure[2] and the resulting 3,4-dicarbethoxy-2-furanpropanol (III) was saponified and the corresponding dicarboxylic acid acetylated to

(1) The authors wish to express their appreciation to Ciba Pharmaceutical Products, Inc., The Research Corporation, New York, and the Buhl Foundation, for grants supporting this work.

(2) Hofmann, THIS JOURNAL, **67**, 421 (1945).

(3) Hofmann and Bridgwater, *ibid.*, **67**, 738 (1945).

(4) Hofmann and Bridgwater, *ibid.*, **67**, 1165 (1945).

(5) Hofmann, *ibid.*, **67**, 1459 (1945).

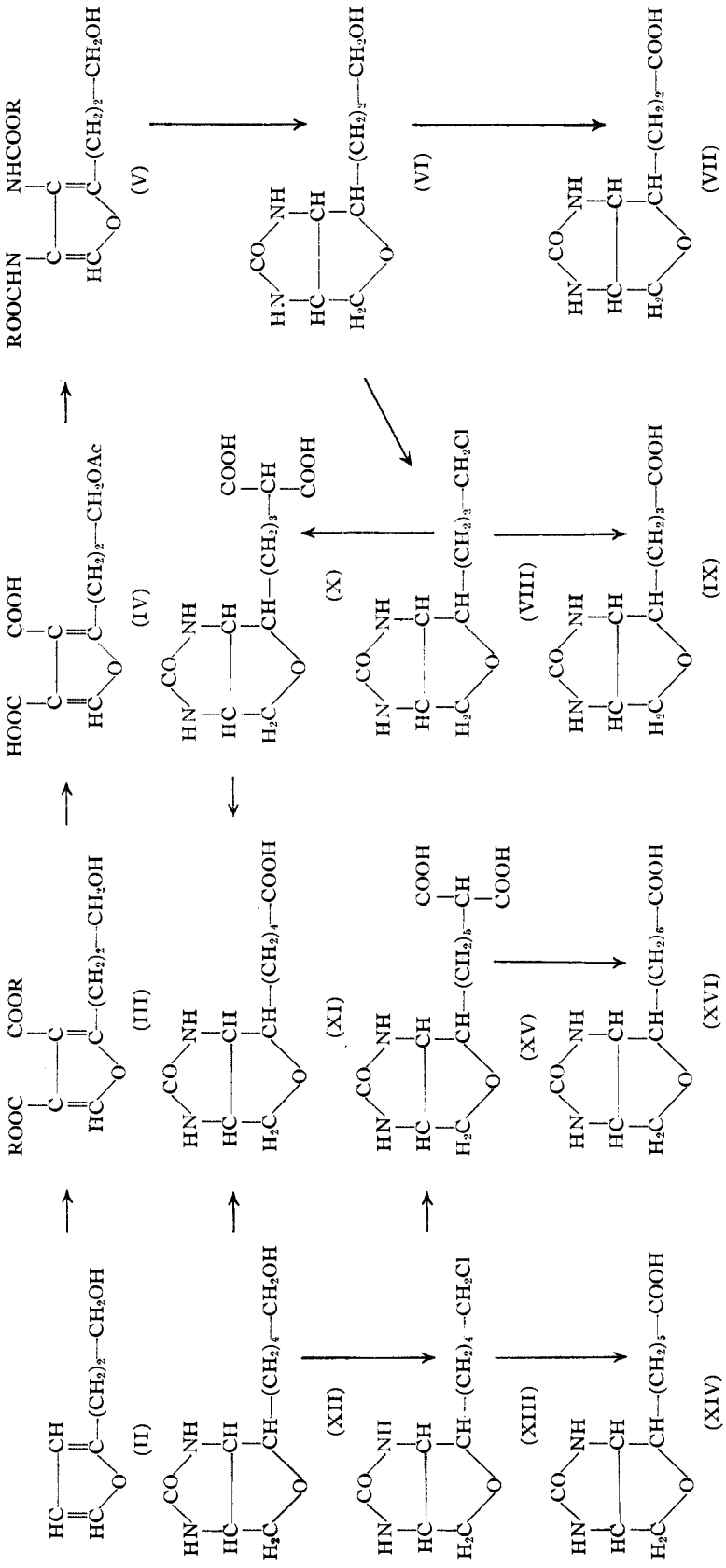
(6) Pilgrim, Axelrod, Winnick and Hofmann, *Science*, **102**, 35 (1945).

(7) Rubin, Flower, Rosen and Drecter, *Arch. Biochem.*, **8**, 79 (1945).

(8) Hofmann and Winnick, *J. Biol. Chem.*, **160**, 449 (1945).

(9) Axelrod, Flinn and Hofmann, unpublished observations.

(10) Bray and Adams, THIS JOURNAL, **49**, 2101 (1927).



form (IV). Degradation of (IV) by a modified Curtius procedure,[3] followed by partial saponification, gave 3,4-diaminocarbethoxy-2-furanpropanol (V) which was transformed into hexahydro-2-oxo - 4 - (3-hydroxypropyl) - 1 - furo(3,4)imidazole (VI) by catalytic hydrogenation and ring closure with aqueous barium hydroxide as described in previous communications.[4,5] Oxidation of (VI) with potassium permanganate gave *dl*-bis-nor-oxybiotin (VII). *dl*-Nor-oxybiotin (IX) was also prepared from the propanol derivative (VI). It was observed that treatment of the propanol (VI) with an excess of thionyl chloride resulted in the formation of hexahydro-2-oxo-4-(3 - chloropropyl) - 1 - furo(3,4) - imidazole (VIII) without affecting the urea portion of the molecule. Chlorides of this general type are very valuable intermediates for the preparation of a great number of new oxybiotin derivatives. For example, treatment of the chloride (VIII) with potassium cyanide yielded the corresponding nitrile, which on saponification gave *dl*-nor-oxybiotin (IX).

Since it could not be predicted that the catalytic hydrogenation of 3,4-diaminocarbethoxy-2-furanpropanol (V) would result in the same spatial arrangement of the side chain as did the similar reduction of the corresponding pentanol derivative,[5] it became important to prepare *dl*-oxybiotin from the propanol (VI), and thus to correlate the stereochemical relationships of these two compounds in an unambiguous manner. The chloride (VIII) was therefore treated with sodiodiethylmalonate, and the resulting condensation product saponified to yield (X). This compound when heated to 180–190° lost one molecule of carbon dioxide and was transformed into *dl*-oxybiotin (XI). The oxybiotin prepared in this manner was identical in melting point and biological activity with the oxybiotin prepared by our original method.[5] The *dl*-bis-nor-, as well as the *dl*-nor-oxybiotins therefore belong to the

same stereochemical series as oxybiotin. The following reactions were used to prepare the higher oxybiotin homologs. Hexahydro-2-oxo-4-(5-hydroxypentyl)-1-furo(3,4)imidazole[5] (XII) was treated with cold thionyl chloride, and the corresponding chloride (XIII) was obtained. Treatment of this chloride with potassium cyanide followed by alkaline saponification gave *dl*-homo-oxybiotin (XIV) ($n = 5$). On the other hand, elongation of the side chain of (XIII) by means of a malonic ester synthesis resulted in the formation of the malonic acid derivative (XV), which on heating to 190° lost carbon dioxide to give *dl*-bis-homo-oxybiotin (XVI) ($n = 6$). The spatial configuration of compounds (XIV) and (XVI) must be identical with that of *dl*-oxybiotin (XI) since all three substances are derived from the pentanol (XII).

The microbiological activity of the above-described oxybiotin homologs was determined with *Saccharomyces cerevisiae* and *Lactobacillus arabinosus* as the test organisms. The activity of the oxybiotin homologs is negligible, less than 0.0035% that of oxybiotin. The introduction of a carboxyl group adjacent to the original carboxyl group of oxybiotin (Compound X) causes a thirty-fold diminution in the biological activity for both organisms. This extreme side-chain specificity indicates the significance of the size and shape of the oxybiotin molecule for its biological activity. It may be possible that the intramolecular distance between the carboxyl group and the urea portion is of importance in establishing a configuration necessary for the attachment of the oxybiotin molecule to its specific protein carrier.

In view of the present interest in vitamin antagonists as tools in enzyme studies or as potential chemotherapeutic agents, it was of importance to study the antibiotin and antioxybiotin activity of the oxybiotin homologs. With *S. cerevisiae* and *L. arabinosus* none of the described homologs possessed anti*biotin* activities at a molar inhibition ratio[11] of approximately 500,000. With respect to the anti*oxybiotin* activity, the nor ($n = 3$), the bis-homo ($n = 6$), and the homo analog ($n = 5$), possessed molar inhibition ratios of 143,000, 30,000, and 7400, respectively, with *S. cerevisiae* as the test organism. With this organism the *dl*-bis-nor-oxybiotin ($n = 2$) caused no significant inhibition. With *L. arabinosus* only the homo analog ($n = 5$) possessed comparable antagonistic activity (molar inhibition ratio 225,000). It is of interest that for *S. cerevisiae* this compound is likewise the most potent oxybiotin antagonist in this group. The antagonistic effects of these substances are more pronounced toward *dl*-oxybiotin than toward *d*-biotin, in agreement with our previous observations with other biotin and oxybiotin antagonists.[12]

(11) The ratio of the molar concentration of the antagonists to that of *d*-biotin or *dl*-oxybiotin, at which complete inhibition of growth results.

(12) Axelrod, DeWoody and Hofmann, *J. Biol. Chem.*, **163**, 771 (1946).

Experimental[13,14]

2-Furanpropanol (II).—A solution of 114 g. of freshly distilled furylacrolein in 400 cc. of 95% alcohol was hydrogenated at room temperature and atmospheric pressure in the presence of a Raney nickel catalyst (prepared from 50 g. of the alloy) until an amount of hydrogen corresponding to two moles had been absorbed. The catalyst was removed by filtration through a layer of filter cell, the alcohol evaporated *in vacuo*, and the residue distilled. The fraction boiling at 105–115° at 21 mm., 94 g. (80% of the theoretical yield) was used for further work. This fraction had the following constants: $n^{27}D$ 1.4764, d^{29}_{29} 1.0531.

α-Naphthyl Urethan of 2-Furanpropanol.—This derivative was prepared in the usual manner and was recrystallized from petroleum ether (b. p. 30–60°). Its melting point, 57–59°, did not agree with that given in the literature.[10]

Anal. Calcd. for $C_{18}H_{17}O_3N$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.00; H, 5.81; N, 4.58.

3,4-Dicarbethoxy-2-furanpropanol (II).—A mixture of 77.4 g. of 2-furanpropanol (II) and 105 g. of diethylacetylenedicarboxylate was heated on a steam-bath for twelve hours. The resulting addition compound was dissolved in 300 cc. of ethyl acetate and was hydrogenated in the presence of 14 g. of a palladium on barium sulfate catalyst[15] until one mole of hydrogen was absorbed. The catalyst was removed by filtration, the ethyl acetate removed *in vacuo*, and the residue decomposed *in vacuo* at 190–200° and 12 mm. pressure and then distilled; 119.6 g. (72%) of 3,4-dicarbethoxy-2-furanpropanol (III) was obtained as an oil which boiled at 146–152° at 0.02 mm.

3,4-Dicarboxy-2-furanpropanol.—A solution of 119.6 g. of the above 3,4-dicarbethoxy-2-furanpropanol (III) in 710 cc. of methanol and 355 cc. of 5 *N* potassium hydroxide was refluxed for five hours, after which the methanol was removed *in vacuo*. The residue was diluted with water, acidified with concentrated hydrochloric acid, and the resulting crystals collected, washed with water, and dried over phosphorus pentoxide *in vacuo*. The 3,4-dicarboxy-2-furanpropanol, 90.1 g. (95% of the theoretical yield), was recrystallized from a mixture of ethyl acetate and methanol and melted at 161–162°.

Anal. Calcd. for $C_9H_{10}O_5$: C, 50.47; H, 4.70. Found: C, 50.28; H, 4.79.

3,4-Dicarboxy-2-furanpropanol Acetate (IV).—A mixture of 61.5 g. of the above 3,4-dicarboxy-2-furanpropanol in 255 cc. of dry pyridine and 148 cc. of acetic anhydride was heated on a steam-bath until all of the solid was dissolved, and the resulting solution was kept at room temperature for twelve hours. The solvents were removed *in vacuo*, the residue was acidified to congo red with concentrated hydrochloric acid, and the crude acetate was extracted with ethyl acetate. The material was purified by re-extraction with 10% sodium bicarbonate and acidification; 62.8 g. (85% of the theoretical yield) of the acetate, melting at 70–72°, was obtained. The compound was dried over phosphorus pentoxide *in vacuo* and was used for the next step without further purification.

3,4-Diaminocarbethoxy-2-furanpropanol (V).—To an ice-cold suspension of 36.2 g. of the above 3,4-dicarboxy-2-furanpropanol acetate (IV) in 180 cc. of dry ether, 65.2 g. of powdered phosphorus pentachloride was added slowly with shaking. The mixture was removed from the ice-bath and was shaken at room temperature until all of the material had dissolved. The ether was removed *in vacuo* at a bath temperature of 40° and the resulting sirup redissolved in 100 cc. of dry ether, and evaporated to dryness *in vacuo*. This procedure was repeated twice more in order to remove the excess of phosphorus oxychloride. The resulting acid chloride was dissolved in 360 cc. of ether and the ether solution stirred for two hours in an ice-bath with

(13) All melting points are corrected.

(14) We are indebted to the Quaker Oats Company, Chicago, Ill., for a generous supply of furfural.

(15) Schmidt, *Ber.*, **52**, 409 (1919).

a solution of 36 g. of sodium azide in 100 cc. of water. A forty per cent. solution of potassium hydroxide (73 cc.) was then added and stirring continued for an additional hour. The ether solution was separated from the aqueous layer, washed with ice water, dried over freshly desiccated sodium sulfate, and filtered through a layer of sodium sulfate. The ether was removed *in vacuo* at room temperature, the oily azide dissolved in 360 cc. of absolute alcohol, and decomposed under nitrogen at 40–50° as described in previous publications.[4,5] The resulting alcoholic solution of 3,4-diaminocarbethoxy-2-furanpropanol acetate was cooled to room temperature, and 142 cc. of *N* sodium hydroxide slowly added with stirring. The mixture was kept at room temperature overnight, the alcohol removed *in vacuo*, and the residue dissolved in ether. The ether solution was washed with three portions of 2 *N* hydrochloric acid and one portion of 10% sodium bicarbonate, dried over sodium sulfate, and the ether removed *in vacuo*. The resulting oil solidified in a short time. Seventy-six grams of (IV) in all was treated in this manner and after recrystallization from 60% aqueous methanol yielded 30 g. (33% of the theoretical yield) of 3,4-diaminocarbethoxy-2-furanpropanol (V), m. p. 74–77°.

Anal. Calcd. for $C_{13}H_{20}O_6N_2$: C, 52.00; H, 6.71; N, 9.33. Found: C, 52.03; H, 6.60; N, 9.62.

***dl*-Hexahydro-2-oxo-4-(3-hydroxypropyl)-1-furo(3,4)-imidazole (VI).**—Six grams of the above 3,4-diaminocarbethoxy-2-furanpropanol (V) was dissolved in 200 cc. of glacial acetic acid, and hydrogenated over 6 g. of a palladium on barium sulfate[15] catalyst. An amount of hydrogen corresponding to two moles was absorbed in approximately four hours, when the hydrogen uptake came to an end. The catalyst was removed by filtration, the solvent removed *in vacuo*, the resulting oil dissolved in 400 cc. of a 10% solution of barium hydroxide, and the mixture heated at 90° for two hours with stirring. A stream of carbon dioxide was passed into the hot solution for approximately thirty minutes, the barium carbonate removed by filtration, and the resulting yellow solution evaporated to dryness *in vacuo*. The gummy residue was extracted with three 50-cc. portions of dioxane which were combined and concentrated to a small volume *in vacuo*. The residue was kept at room temperature until crystallization occurred. The crystals were collected and dried over phosphorus pentoxide *in vacuo;* 1.4 g. (37% of the theoretical yield) of crude material was obtained. Recrystallization from a mixture of dioxane and methanol gave prisms which melted at 137–139°.

Anal. Calcd. for $C_8H_{14}O_3N_2$: C, 51.60; H, 7.58; N, 15.04. Found: C, 51.85; H, 7.69; N, 15.24.

***dl*-Hexahydro-2-oxo-1-furo(3,4)imidazole-4-propionic Acid (VII) (*dl*-bisnor-Oxybiotin).**—Nine hundred mg. of the above hexahydro-2-oxo-4-(3-hydroxypropyl)-1-furo-(3,4)imidazole (VI) was oxidized with a 5% solution of potassium permanganate as described for the preparation of oxybiotin.[5] The resulting *dl*-bisnor-oxybiotin, 300 mg. (31% of the theoretical yield), was purified by recrystallization from water. Short prisms melting at 202–204° were obtained.

Anal. Calcd. for $C_8H_{12}O_4N_2$: C, 48.00; H, 6.04; N, 13.99. Found: C, 48.00; H, 6.00; N, 13.95.

***dl*-Hexahydro-2-oxo-4-(3-chloropropyl)-1-furo(3,4)-imidazole (VIII).**—One gram of the above hexahydro-2-oxo-4-(3-hydroxypropyl)-1-furo(3,4)imidazole (VI) was placed in a round-bottomed flask surrounded by ice, and 4 cc. of purified thionyl chloride was added. The substance dissolved within a few minutes, and the clear solution was kept at room temperature for twelve hours. The thionyl chloride was then removed *in vacuo* and the resulting oil neutralized with 10% sodium bicarbonate solution, and evaporated to dryness *in vacuo*. The residue was extracted with several portions of hot ethyl acetate which were combined and concentrated to a small volume. The resulting crystals were recrystallized from ethyl acetate; 835 mg. (76% of the theoretical yield) of prismatic crystals was obtained which melted at 122–124°.

Anal. Calcd. for $C_8H_{13}O_2N_2Cl$: C, 46.95; H, 6.40; N, 13.68; Cl, 17.33. Found: C, 47.16; H, 6.39; N, 13.00; Cl, 17.07.

***dl*-Hexahydro-2-oxo-1-furo(3,4)imidazole-4-butyric Acid (IX) (*dl*-nor-Oxybiotin).**—To a solution of 102 mg. of the above chloride (VIII) in two cc. of absolute alcohol was added a solution of 65 mg. of potassium cyanide in 0.2 cc. of water, and the mixture refluxed for twenty-four hours. The solvent was removed *in vacuo* and the residue dissolved in 3 cc. of 1 *N* sodium hydroxide and refluxed for three hours. The solution was acidified to congo red with concentrated hydrochloric acid and evaporated to dryness *in vacuo*. The organic material was separated from inorganic salts by extraction with three 3-cc. portions of absolute alcohol, which were combined and concentrated to dryness *in vacuo*. The resulting *dl*-nor-oxybiotin (33 mg.) was purified by recrystallization from water, and melted at 219–220°.

Anal. Calcd. for $C_9H_{14}O_4N_2$: C, 50.46; H, 6.59; N, 13.07. Found: C, 50.31; H, 6.76; N, 13.32.

***dl*-Hexahydro-2-oxo-4-(4,4-dicarboxybutyl)-1-furo(3,4)-imidazole (X).**—To a solution of 500 mg. of sodium in 16.6 cc. of absolute alcohol, 3.4 cc. of redistilled diethyl malonate was added and the mixture kept at room temperature for thirty minutes. One cc. of this solution was added to a solution of 205 mg. of the above chloride (VIII) in 5 cc. of absolute alcohol, and the mixture refluxed for twelve hours on a steam-bath. The solution was separated from the sodium chloride and the solvent removed *in vacuo*. The residue was dissolved in ethyl acetate and the solution washed with water, dried over sodium sulfate, and the solvent removed *in vacuo*. The residue was dissolved in 5 cc. of 10% aqueous solution of barium hydroxide, and the solution refluxed for two hours. The mixture was acidified with 2 *N* sulfuric acid, and the resulting precipitate of barium sulfate removed by filtration. The filtrate, on concentration to a small volume, deposited crystals of the malonic acid derivative (X) which were collected and purified by crystallization from water; 30 mg. of needles were obtained which melted with decomposition at 180–183°.

Anal. Calcd. for $C_{11}H_{16}O_6N_2$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.66; H, 5.85; N, 10.41.

***dl*-Hexahydro-2-oxo-1-furo(3,4)imidazole-4-valeric Acid (XI) (*dl*-Oxybiotin).**—Thirty mg. of the above malonic acid derivative (X) was heated in a small test-tube to 180–190° until the evolution of carbon dioxide came to an end. The resulting *dl*-oxybiotin was recrystallized from 95% alcohol and melted at 203–204°. No depression of the melting point was observed when the material was admixed with a sample of oxybiotin, prepared according to our original procedure.[5]

Anal. Calcd. for $C_{10}H_{16}O_4N_2$: C, 52.62; H, 7.07; N, 12.27. Found: C, 53.02; H, 7.58; N, 11.98.

***dl*-Hexahydro-2-oxo-4-(5-chloropentyl)-1-furo(3,4)imidazole (XIII).**—The pentanol (XII)[5] (1.35 g.) was treated with 5 cc. of thionyl chloride as described for the preparation of the corresponding propyl chloride (VIII). The thionyl chloride was removed *in vacuo*, the residue neutralized with 10% sodium bicarbonate, and the mixture extracted with ethyl acetate. The ethyl acetate solution was washed with sodium bicarbonate and water, dried over sodium sulfate and concentrated to a small volume; 900 mg. (62% of the theoretical yield) of the desired chloride separated from the solution in short prisms which melted at 93–94°. A sample for analysis was recrystallized from carbon tetrachloride.

Anal. Calcd. for $C_{10}H_{17}O_2N_2Cl$: C, 51.61; H, 7.37; N, 12.03; Cl, 15.24. Found: C, 51.33; H, 7.16; N, 12.71; Cl, 15.59.

***dl*-Hexahydro-2-oxo-1-furo(3,4)imidazole-4-caproic Acid (XIV) (*dl*-Homo-oxybiotin).**—To a solution of 166 mg. of the above chloride (XIII) in 5 cc. of absolute alcohol was added 65 mg. of potassium cyanide dissolved in 0.2 cc. of water, and the mixture refluxed for twenty-four hours. The solvents were removed *in vacuo* and the

residue hydrolyzed by refluxing for three hours with 3 cc. of 1 *N* sodium hydroxide. Acidification of the solution with concentrated hydrochloric acid gave 98 mg. of needles which were recrystallized from water. The purified product melted at 207–208°.

Anal. Calcd. for $C_{11}H_{18}O_4N_2$: C, 54.53; H, 7.49; N, 11.56. Found: C, 54.65; H, 7.42; N, 11.87.

***dl*-Hexahydro-2-oxo-4-(6,6-dicarboxyhexyl)-1-furo(3,4) imidazole (XV).**—To a solution of 233 mg. of the above chloride (XIII) in 5 cc. of absolute alcohol, one cc. of an ethyl malonate solution was added which was prepared as described for the preparation of (X), and the solution refluxed for twelve hours. The solvent was removed *in vacuo*, the residue dissolved in ethyl acetate, and the solution washed with water, dried over sodium sulfate, and the solvent removed *in vacuo*. The resulting oil was dissolved in 5 cc. of a 10% aqueous solution of barium hydroxide, and the solution heated on a steam-bath for two hours. The mixture was acidified with 2 *N* sulfuric acid and the barium sulfate removed by filtration. The filtrate was concentrated to a small volume *in vacuo* and the malonic acid (XV) which separated out was purified by recrystallization from water; 114 mg. of material, melting with decomposition at 175–176°, was obtained.

Anal. Calcd. for $C_{13}H_{20}O_6N_2$: C, 51.99; H, 6.71; N, 9.32. Found: C, 52.34; H, 6.79; N, 9.47.

***dl*-Hexahydro-2-oxo-1-furo(3,4)imidazole-4-heptoic Acid (XVI) (*dl*-Bis-homo-oxybiotin).**—Forty-five mg. of the above malonic acid (XV) was decarboxylated in a small test-tube at a temperature of 180–190° and the resulting *dl*-bis-homo-oxybiotin purified by recrystallization from water. Needles melting at 184–185° were obtained.

Anal. Calcd. for $C_{12}H_{20}O_4N_2$: C, 56.24; H, 7.87; N, 10.93. Found: C, 56.33; H, 7.88; N, 10.59.

Biochemical Experiments

The growth-promoting activities of the compounds for *S. cerevisiae* and *L. arabinosus* were determined as described previously.[16] The molar inhibition ratios[11] were calculated from the minimal amounts of substance necessary to completely inhibit growth in the presence of 0.1 millimicrogram (mγ) of *d*-biotin or 0.2 mγ of *dl*-oxybiotin for *L. arabinosus*, and 0.1 mγ of *d*-biotin and 0.4 mγ of *dl*-oxybiotin for *S. cerevisiae*.

Acknowledgment.—The microanalyses reported in this paper were performed in our microanalytical laboratories by Mr. George L. Stragand. The authors wish to express their appreciation to Barbara Flinn, Jean DeWoody, and Audrey Ransom for technical assistance.

Summary

A number of oxybiotin homologs with the same stereochemical configuration as *dl*-oxybiotin have been synthesized. These homologs were tested both as growth promoters and as growth inhibitors for *S. cerevisiae* and *L. arabinosus*. None of the homologs had significant growth-promoting activity, indicating a high degree of specificity of the valeric acid side chain in oxybiotin. Some of the compounds, especially *dl*-homo-oxybiotin, had inhibitory activity which was more pronounced toward oxybiotin than toward biotin.

(16) Winnick, Hofmann, Pilgrim and Axelrod, *J. Biol. Chem.*, **161**, 405 (1945).

PITTSBURGH, PENNSYLVANIA RECEIVED AUGUST 17, 1946

[CONTRIBUTION FROM THE WYETH INSTITUTE OF APPLIED BIOCHEMISTRY]

Purification of Streptomycin Salts by Means of Alumina[1]

BY GEORGE P. MUELLER

Streptomycin obtained from broths by adsorption on charcoal at *p*H 7 to 8, and subsequent elution, varies in potency according to the procedure employed, but generally lies between 150 and 450 γ/mg.[2,3] Carter, *et al.*, have described the purification of 200-γ streptomycin hydrochloride up to a potency of 800 γ/mg. by chromatographic adsorption on acid-washed alumina.[2] These experiments have been repeated in this Laboratory with results that are in excellent agreement with the data published.

Although yields are good and certain fractions of the eluate from such alumina columns yield streptomycin of the highest potency, the application of chromatography to large-scale purifications is, as always, beset with the necessity of working with large quantities of solvents and adsorbent. In order to find a method suitable for the purification of large quantities, it was considered possible to make use of selective adsorption by the alumina of the impurities from aqueous-methanolic solutions of crude streptomycin. This behavior was characteristic of the acid-washed alumina columns.

A method is presented here in which streptomycin concentrates of potencies 290 and 360 γ/mg. may be increased by any amount in keeping with the yield desired, within a practical upper limit of 650 γ/mg. The success of this method depends upon the extent of selective adsorption of impurities, and conditions under which this selectivity may be increased or decreased are described. Besides being economical with solvents and adsorbent, this scheme is easily adapted to large quantities of relatively low-grade streptomycin concentrates and permits a prediction of the yield and potency of the product from any operation, once the characteristics of the adsorbent are known.

In seeking this method, initial trials were made by filtering solutions of impure streptomycin hydrochloride through thin beds of the adsorbent. Decoloration and about 30% increase in potency were obtained with beds only 2 mm. thick. Usually, however, channelling of these thin beds

(1) This paper was presented before the Division of Biological Chemistry at the American Chemical Society Meeting in Chicago, 1946.

(2) Carter, Clark, Dickman, Loo, Skell and Strong, *J. Biol. Chem.*, **160**, 337 (1945).

(3) LePage and Campbell, *ibid.*, **162**, 163 (1946).