

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS &amp; COMPANY, DETROIT]

Chloromycetin (Chloramphenicol<sup>1</sup>) Related Compounds: Some Tertiary Alcohols

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RECEIVED SEPTEMBER 4, 1952

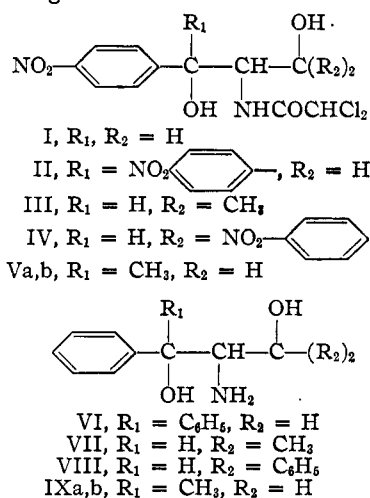
The antibiotic substance Chloromycetin has been chemically designated as *DL-threo*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol. The preparation of a series of compounds in which the "1" hydrogen or the two "3" hydrogens of the above substance have been replaced by *p*-nitrophenyl or methyl is described.

A number of compounds related to Chloromycetin (I) but differing from the antibiotic in the structure of the side chain have been reported. These modifications include compounds in which one or both of the hydroxyls are replaced by hydrogen or alkyl,<sup>2</sup> compounds in which the side chain is extended by substitution of a methyl or *p*-nitrophenyl group in the "2" or "3" position,<sup>3,4</sup> the dichloroacetamides of *DL-erythro*- and *DL-threo*-*p*-nitrophenylserine and esters of these compounds<sup>5,6-10</sup> and 2-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol.<sup>11</sup>

In the compounds to be described the hydrogen atom or atoms attached to the carbon at the "1" or "3" position of the Chloromycetin side chain have been replaced by *p*-nitrophenyl or methyl groups. The alcohol group attached to this carbon thus becomes tertiary. Interest in the preparation of compounds II and IV was first stimulated by a report that 2,2,2-trichloro-1,1-bis-(nitrophenyl)-ethane, an analog of DDT, is effective in the treatment of experimental murine typhus in mice.<sup>12</sup> Additional motivation for the study of these compounds was, of course, the fact that Chloromycetin is not only effective in similar tests but an extremely practical agent for treating typhus fever in humans as first demonstrated by Payne, Knaudt and Palacios.<sup>13</sup>

Intermediates useful in the synthesis of compound II were obtained by treating serine ester hydrochloride or  $\alpha$ -amino- $\beta$ -hydroxypropiophenone with phenylmagnesium bromide. The product in each case was 1,1-diphenyl-2-amino-1,3-propanediol (VI). Intermediates for compounds III and IV were similarly prepared by treating *DL-threo*-phenylserine ester hydrochloride with methylmagnesium bromide or phenylmagnesium bromide to give 1-phenyl-2-amino-3,3-diphenyl or (dimethyl)-1,3-propanediol (VII and VIII). The ben-

zamides of the diastereoisomeric compounds IXa and IXb were obtained by the reaction of  $\alpha$ -benzamido- $\beta$ -hydroxypropiophenone with the methyl Grignard reagent.



With the exception of the latter diastereoisomers, the products of the Grignard reactions were isolated as free bases (VI, VII and VIII). The bases were in turn converted to dichloroacetamides and acetylated by treatment with pyridine-acetic anhydride mixtures at room temperature. Although the tertiary hydroxyl groups were not acetylated under these conditions, the products could be nitrated by treatment with fuming nitric acid without complication. Following nitration the acetoxy group was removed by mild alkaline hydrolysis and the desired products II, III and IV were isolated.

Since the phenylserine used in the preparation of 1-phenyl-2-amino-3,3-dimethyl or (diphenyl)-1,3-propanediol has been shown to have the *threo* configuration<sup>14</sup> and since the subsequent reactions used to convert these products to the 1-*p*-nitrophenyl-2-dichloroacetamides presumably do not cause inversion, compounds III and IV are thought to belong to the *threo* series.

When  $\alpha$ -benzamido- $\beta$ -hydroxypropiophenone was treated with methylmagnesium bromide both the *erythro* and *threo* diastereoisomers were produced. Although the resulting mixture of benzamides did not crystallize readily, acetylation gave derivatives which could be separated by fractional crystallization. The individual isomers were nitrated, the benzoyl and acetyl groups subsequently removed by acid hydrolysis, and the free bases converted to the diastereoisomeric dichloroacetamides of 1-*p*-nitrophenyl-1-methyl-2-amino-1,3-

- (1) Chloramphenicol is the generic name for the antibiotic identified as Chloromycetin, a Parke, Davis & Company trademark.
- (2) M. C. Rebstock, G. W. Moersch, A. C. Moore and J. M. Vandenberg, *THIS JOURNAL*, **73**, 3666 (1951).
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- (13) C. H. Payne, J. A. Knaudt and S. Palacios, *J. Trop. Med.*, **51**, 681 (1948).
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propanediol. It was not determined which of the isomers was *threo* and which *erythro*.

In biological studies to be reported elsewhere none of the above compounds was found to possess significant antibacterial, antirickettsial or anti-virus properties.<sup>15</sup>

### Experimental

**DL-1,1-Diphenyl-2-amino-1,3-propanediol (VI). Method A.**—To a well-stirred solution of phenylmagnesium bromide prepared from 64 g. of magnesium turnings and 400 g. of bromobenzene in 1 l. of absolute ether was added 42 g. of DL-serine ethyl ester hydrochloride. The addition which was regulated by the reflux rate required 3 to 4 hr. After refluxing overnight the reaction mixture was poured on ice to which ammonium chloride had been added. The quench mixture was separated and the aqueous phase extracted twice with ethyl acetate. Evaporation of the combined extracts gave a gummy residue which was taken into 1:2 dilute hydrochloric acid and extracted with ether, the ether extract being discarded. Chilling of the aqueous layer gave a white solid m.p. 240–242° dec., which dried to a weight of 27.5 g. The filtrate was adjusted to pH 10 with aqueous sodium hydroxide, then extracted successively with ether and ethyl acetate. Evaporation of the extract gave an oil which was again dissolved in dilute hydrochloric acid, treated with Norite, filtered and chilled. This second crop of 9.5 g. melted poorly at ca. 200°. A sample of the first crop recrystallized from water melted at 252–253° dec.

*Anal.*<sup>16</sup> Calcd. for  $C_{15}H_{15}O_2NCl$ : C, 64.39; H, 6.48; N, 5.01. Found: C, 64.46; H, 6.48; N, 5.12.

**Method B.**—To a well-stirred solution of phenylmagnesium bromide, prepared from 30 g. of magnesium turnings and 200 g. of bromobenzene in 500 ml. of absolute ether was added 40 g. of  $\alpha$ -amino- $\beta$ -hydroxypropiophenone hydrochloride. The solid was added in portions at a rate determined by the reflux rate. After standing overnight at room temperature the mixture was quenched on ice and the ether layer decanted. By careful addition of dilute hydrochloric acid to the aqueous phase the gelatinous precipitate was dissolved, the pH remaining on the basic side. The clear solution was extracted twice with ethyl acetate and the combined ether and ethyl acetate extracts concentrated *in vacuo*. Soon after dissolving the residual oil in dilute hydrochloric acid, white crystals separated. These were removed by filtration and desiccated to a weight of 30 g. (m.p. 252–253°). A second crop was obtained by chilling the filtrate (yield 6 g., m.p. ca. 215°).

The free base was obtained by dissolving 22 g. of the above hydrochloride in 750 ml. of water and adding aqueous ammonia to ca. pH 10. The oil which separated soon solidified to give 15 g. of product which was recrystallized from 25% aqueous ethanol to a melting point of 98–99°. A second recrystallization from water gave the pure base melting at 103°.

*Anal.* Calcd. for  $C_{15}H_{17}O_2N$ : C, 74.04; H, 7.05; N, 5.76. Found: C, 74.09; H, 7.14; N, 5.59.

The diacetyl derivative was prepared by heating 2.0 g. of the base with 10 ml. of acetic anhydride for 30 min. on the steam-bath. The mixture was chilled and diluted with ether to precipitate the product. After recrystallization from ethanol, the derivative melted at 193–194°.

*Anal.* Calcd. for  $C_{19}H_{21}NO_4$ : C, 69.70; H, 6.47; N, 4.28. Found: C, 69.64; H, 6.32; N, 4.32.

Hydrolysis of 2 g. of 1,1-diphenyl-2-acetamido-1,3-propanediol acetate ester in the presence of 25 ml. of ethanol containing 0.5 ml. of 10% aqueous sodium hydroxide gave 1,1-diphenyl-2-acetamido-1,3-propanediol which melted at 197°.

*Anal.* Calcd. for  $C_{17}H_{19}NO_3$ : C, 71.56; H, 6.71; N, 4.91. Found: C, 71.66; H, 6.60; N, 4.80.

(15) We are indebted to Dr. I. W. McLean and Mr. Frank Miller for the antiviral and antirickettsial studies and to Dr. J. Ehrlich, Dr. A. S. Schlingman, Mrs. Della Fox, Miss M. E. Doles and Miss M. Manning for the antibacterial tests.

(16) We wish to acknowledge our indebtedness to Mr. C. E. Childs, Mrs. Geraldine Koch and Miss Virginia Pawlik for the many micro-analytical determinations.

**DL-threo-1-Phenyl-2-dichloroacetamido-3,3-dimethyl-1,3-propanediol.**—A solution of methylmagnesium bromide was prepared by adding 116.5 g. of methyl bromide dissolved in 250 ml. of anhydrous ether to 29.5 g. of magnesium turnings suspended in 250 ml. of ether. To the Grignard reagent was added in portions 25 g. of the ethyl ester of phenylserine hydrochloride at a rate permitting the solution to reflux gently. Forty minutes was required for the addition. The reaction mixture was refluxed for two hours longer and then allowed to stand at room temperature overnight. The contents of the flask were poured on ice and extracted with ether. The aqueous residue was partially neutralized with 5 N sulfuric acid to facilitate extraction and the still basic mixture extracted three times with ethyl acetate. The combined ether and acetate extracts were dried over magnesium sulfate and evaporated. The gummy residue was dissolved in ethylene dichloride and kept overnight in the refrigerator. A crystalline product which amounted to 1.25 g. after recrystallization from ethyl acetate and which melted at 149–150° was isolated. This compound which was not the desired base has not been identified. The ethylene dichloride mother liquor was evaporated and the gummy residue converted without purification to the dichloroacetamide by refluxing with methyl dichloroacetate in ethanol for 1 hr. The solvents were evaporated at reduced pressure, the residue triturated with low boiling petroleum ether to remove excess ester and then taken into 200 ml. of ethyl acetate. The acetate solution was washed twice with 0.1 N sulfuric acid which removed considerable color and then with water, following which it was dried and evaporated. A yield of 5.3 g. of crystalline product melting at 152–155° was obtained after recrystallization from benzene. An analytical sample prepared by recrystallizing from chloroform and finally from ethylene dichloride melted at 157–158°.

*Anal.* Calcd. for  $C_{13}H_{17}NO_3Cl_2$ : C, 50.99; H, 5.68; N, 4.58. Found: C, 50.71; H, 5.80; N, 4.70.

**DL-threo-1-Phenyl-2-amino-3,3-diphenyl-1,3-propanediol (VIII).**—This product was obtained by treatment of 25 g. of DL-threo-phenylserine ester hydrochloride with phenylmagnesium bromide using conditions essentially identical with those described in the preceding example. Recrystallization from ethanol gave 22 g. of product which melted at 154–155° after two further recrystallizations.

*Anal.* Calcd. for  $C_{21}H_{23}NO_2$ : C, 78.97; H, 6.63; N, 4.38. Found: C, 79.00; H, 6.74; N, 4.33.

**DL-threo- and DL-erythro-1-Phenyl-1-methyl-2-benzamido-1,3-propanediol-3-acetate Ester.**—Fifty grams of  $\alpha$ -benzamido- $\beta$ -hydroxypropiophenone was added to ten equivalents of methylmagnesium iodide in ether solution in the usual manner. Since the product did not crystallize readily it was acetylated by dissolving in a mixture of 75 ml. of pyridine and 100 ml. of acetic anhydride. After standing overnight at room temperature the reaction mixture was poured into ice-water and the product extracted with ether. The combined extracts were dried and evaporated and the residue crystallized from ether to give 40 g. of product which consisted of a mixture of the diastereoisomers. Recrystallization from aqueous ethanol resulted in the separation of one isomer in substantially pure state. The melting point could be raised from 138–141° to 145–146° by recrystallization from ethyl acetate, benzene and finally ethyl acetate. The other isomer was obtained by recrystallization of the residue isolated from the mother liquor first from ether and finally three times from ethyl acetate. The product melted at 113–114°. Extensive purification of the low melting isomer was found to be unnecessary as the crude material could be nitrated and taken through the remaining steps of the synthesis to yield a pure final product.

*Anal.* Calcd. for  $C_{19}H_{21}NO_4$ : C, 69.71; H, 6.47; N, 4.28. Found (isomer melting at 145–146°): C, 69.47; H, 6.17; N, 4.52. Found (isomer melting at 113–114°): C, 69.86; H, 6.48; N, 4.32.

**Dichloroacetamides and Acetate Esters of Dichloroacetamides of VI, VII and VIII.**—The dichloroacetamides were prepared by refluxing the free bases in alcohol solution in the presence of excess methyl dichloroacetate. The products were isolated by evaporation of the alcohol, trituration with low boiling petroleum ether to remove excess ester and finally extraction into ethyl acetate. The ethyl acetate solutions were washed with 0.1 N sulfuric acid, saturated sodium bi-

TABLE I

		M.p., °C.	Formula	Carbon, % Calcd. Found	Hydrogen, % Calcd. Found	Nitrogen, % Calcd. Found
Dichloroacetamides of phenylaminopropanediols						
(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub> COH—CHNH <sub>2</sub> —CH <sub>2</sub> OH	VI	146	C <sub>17</sub> H <sub>17</sub> NO <sub>3</sub> Cl <sub>2</sub>	57.64 57.41	4.84 4.92	3.95 4.10
DL-C <sub>6</sub> H <sub>5</sub> —C(OH)(H)—C(NH <sub>2</sub> )(COH)(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>	VIII	208-210	C <sub>23</sub> H <sub>21</sub> NO <sub>3</sub> Cl <sub>2</sub>	64.19 64.04	4.92 4.92	3.26 3.39
Acetate esters of dichloroacetamides						
(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub> COH—CHNH <sub>2</sub> —CH <sub>2</sub> OH	VI	142-143	C <sub>19</sub> H <sub>11</sub> NO <sub>4</sub> Cl <sub>2</sub>	57.59 57.63	4.83 5.06	3.54 3.73
DL-C <sub>6</sub> H <sub>5</sub> —C(OH)(H)—C(NH <sub>2</sub> )(COH)(CH <sub>3</sub> ) <sub>2</sub>	VII	167-169	C <sub>15</sub> H <sub>19</sub> NO <sub>4</sub> Cl <sub>2</sub>	51.73 51.98	5.40 5.46	4.02 3.96
DL-C <sub>6</sub> H <sub>5</sub> —C(OH)(H)—C(NH <sub>2</sub> )(COH)(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>	VIII	165-167	C <sub>25</sub> H <sub>23</sub> NO <sub>4</sub> Cl <sub>2</sub>	63.57 63.45	4.91 5.11	2.97 3.09

TABLE II<sup>a</sup>

		M.p., °C.	Formula	Carbon, % Calcd. Found	Hydrogen, % Calcd. Found	Nitrogen, % Calcd. Found
a <sup>b</sup>	( <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> COH—CH—CH <sub>2</sub> OH   NHCOCHCl <sub>2</sub> II	172-173	C <sub>17</sub> H <sub>15</sub> O <sub>7</sub> N <sub>3</sub> Cl <sub>2</sub>	45.96 46.08	3.40 3.55	9.46 9.42
b	( <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> COH—CH—CH <sub>2</sub> OCOCH <sub>3</sub>   NHCOCH <sub>3</sub>	225	C <sub>19</sub> H <sub>29</sub> N <sub>3</sub> O <sub>8</sub>	54.67 54.72	4.59 4.56	10.07 10.00
c	( <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> COH—CH—CH <sub>2</sub> OH   NHCOCH <sub>3</sub>	214-215	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>7</sub>	54.40 54.58	4.57 4.81	
d	DL- <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> —C(OH)(H)—C(NHCOCHCl <sub>2</sub> )(COH)(CH <sub>3</sub> ) <sub>2</sub>   H III	194-196	C <sub>15</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub> Cl <sub>2</sub>	44.46 44.78	4.59 4.80	7.98 7.96
e	DL- <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> —C(OH)(H)—C(NHCOCHCl <sub>2</sub> )(COH)(C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub> - <i>p</i> ) <sub>2</sub>   H IV	236-238	C <sub>25</sub> H <sub>18</sub> N <sub>4</sub> O <sub>9</sub> Cl <sub>2</sub>	48.86 48.69	3.21 3.35	9.91 9.83
f	DL- <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> —C(OH)(CH <sub>3</sub> )(NHCOCHCl <sub>2</sub> )—CH—CH <sub>2</sub> OH   OH Va	135-136	C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub> Cl <sub>2</sub>	42.76 43.02	4.18 4.42	8.31 8.35
g	DL- <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> —C(OH)(CH <sub>3</sub> )(NHCOCHCl <sub>2</sub> )—CH—CH <sub>2</sub> OH   OH Vb	172-173	C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub> Cl <sub>2</sub>	42.76 42.58	4.18 4.36	8.31 8.25
h	DL- <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> —C(OH)(CH <sub>3</sub> )—CHNH <sub>2</sub> —CH <sub>2</sub> OH   OH a	151-152	C <sub>16</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	53.08 53.31	6.24 6.07	12.38 12.27
i	DL- <i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> —C(OH)(CH <sub>3</sub> )—CHNH <sub>2</sub> —CH <sub>2</sub> OH   CH <sub>3</sub> b	147-148	C <sub>16</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	53.08 53.19	6.24 6.40	12.38 12.21

<sup>a</sup> Solvents used in the recrystallization of compounds in Table II were: a, aqueous ethanol; b, methanol, then ethanol; c, aqueous methanol; d, ethylene dichloride, ethyl acetate, and finally ethanol; e, ethylene dichloride; f, ethylene dichloride; g, ethylene dichloride and finally ethyl acetate; h, ethylene dichloride and finally ethanol; i, ethyl acetate. <sup>b</sup> *p*-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>— refers to the *p*-nitrophenyl radical.

carbonate solution and water. After drying over magnesium sulfate the solvent was removed at reduced pressure. The products were recrystallized and finally acetylated by treatment with excess 1:1 pyridine-acetic anhydride mixtures at room temperature for 24 hours. The acetylating reagents were removed *in vacuo* and the acetoxy derivatives purified by recrystallization.

**Nitration of the Acetate Ester-Dichloroacetamides of VI, VII and VIII and the 1-Phenyl-1-methyl-2-benzamido-1,3-propanediol-3-acetate Ester Diastereoisomers.**—Each of the above compounds was added in portions during 15 min. to fuming nitric acid. Three milliliters of acid per gram of solid was used and the temperature of the reaction mixture kept at -20° by adding Dry Ice during the addition of the aromatic compound. The nitration mixtures were allowed

to stand for 30 min. longer at room temperature before quenching on ice. The solid products were removed by filtration and dissolved in ethyl acetate. The ethyl acetate solutions were washed with saturated NaHCO<sub>3</sub> until neutral, then dried and evaporated. The 1,1-bis-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol-3-acetate ester was recrystallized directly from ethyl acetate-low boiling petroleum ether (without the bicarbonate wash) to give a product melting at 203-204°. One additional recrystallization raised the melting point to 210°.

*Anal.* Calcd. for C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>8</sub>Cl<sub>2</sub>: C, 46.92; H, 3.52; N, 8.64. Found: C, 47.33; H, 3.76; N, 8.65.

1-*p*-Nitrophenyl-2-dichloroacetamido-3,3-bis-*p*-nitrophenyl-1,3-propanediol-1-acetate ester was recrystallized first

from ethylene dichloride and finally an ethyl acetate-acetone-low boiling petroleum ether mixture (m.p. 246–248°).

Anal. Calcd. for  $C_{25}H_{20}N_4O_{10}Cl_2$ : C, 49.44; H, 3.32. Found: C, 49.65; H, 3.64.

The compounds 1-*p*-nitrophenyl-2-dichloroacetamido-3,3-dimethyl-1,3-propanediol-1-acetate ester and the 1-*p*-nitrophenyl-1-methyl-2-benzamido-1,3-propanediol-3-acetate ester diastereoisomers were used directly without purification.

**Conversion to Products II, III, IV and V.**—The acetyl groups of dichloroacetamido-acetate esters were selectively removed by treatment with an excess of dilute sodium hy-

droxide in aqueous acetone solution. The *p*-nitrobenzamides were hydrolyzed by heating on the steam-bath with excess 10% aqueous ethanolic hydrochloric acid for 3 hr. The hydrolysates were chilled and extracted with ether. The aqueous residue was made alkaline with ammonia and the free base extracted into ethyl acetate. Four such extracts were combined and evaporated. The products were recrystallized from appropriate solvents and converted to the dichloroacetamides by refluxing with an alcoholic solution of methyl dichloroacetate as described above.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

## Isolation of the *Lactobacillus bulgaricus* Factor from Natural Sources<sup>1</sup>

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RECEIVED JUNE 12, 1952

Procedures for the isolation of one form of the *Lactobacillus bulgaricus* factor (LBF-1A) from the culture filtrate of *Ashbya gossypii* are described. These involve adsorption and elution from activated carbon and subsequent successive chromatography on Floridin, carbon, Superfilitrol and alumina. With minor modifications, the procedures are applicable to purification of other forms of LBF from natural materials and to purification of synthetic pantetheine from crude reaction mixtures.

The discovery and partial purification of a widely distributed, unidentified growth factor for *Lactobacillus bulgaricus* was first reported in 1947.<sup>3,4</sup> Subsequent work showed that the same growth factor (termed LBF, or *Lactobacillus bulgaricus* factor) was required by many different lactic acid bacteria,<sup>4,5</sup> that it occurred naturally in several chromatographically distinct forms<sup>6</sup> and that several microorganisms that grew in its absence secreted it into the medium.<sup>6</sup> More recently, pantothenic acid was found<sup>7–9</sup> to have low LBF activity, and digestion of coenzyme A with intestinal phosphatase was shown to produce LBF as one degradation product.<sup>7,10</sup> Finally, the synthesis of two active products, termed pantetheine and pantethine, by condensation of methyl pantothenate with  $\beta$ -mercaptoethylamine was achieved.<sup>11</sup>

(1) Supported in part by grants from the National Institutes of Health (RG 738), Merck and Co., Inc., Parke, Davis and Co., and the Research Committee of the Graduate School from funds provided by the Wisconsin Alumni Research Foundation. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. We are indebted to Drs. J. M. Van Lanen and Karl Smiley of Hiram Walker and Sons, Inc., Peoria, Illinois, who supplied specially fermented culture filtrates as source materials for fractionation, and who kindly carried through preliminary concentration steps on a pilot plant scale according to our directions. Parke, Davis and Co. (thanks are due particularly to Dr. O. D. Bird) cooperated in a similar manner during the latter phases of the investigation. Mrs. Jean A. Craig conscientiously carried out most of the countless microbiological assays involved in this study.

(2) Department of Chemistry, University of Texas, Austin, Texas.

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Exhaustive tests against twenty-odd microorganisms<sup>9</sup> indicated that pantetheine and one of the naturally-occurring forms of LBF (LBF-1A) possessed identical growth-promoting properties; as shown later, however, the two differ in that LBF-1A (as well as several other naturally-occurring forms of LBF) is a mixed disulfide formed by oxidation of pantetheine in the presence of an inert mercaptan.<sup>10</sup>

This paper describes the procedures used in isolation of LBF-1A, one of the forms of LBF that occurs in culture filtrates of *Ashbya gossypii*.<sup>6</sup> A summary of the procedures used is shown in Fig. 1; details of the various steps are described in the Experimental portion. Concentrates obtained in this way provided the material used in elucidation of the chemical nature of the growth factor.<sup>11,12</sup> Subsequently, these procedures also proved applicable to isolation of the growth factor from synthetic reaction mixtures.<sup>11,13</sup> Since these experiments were completed another procedure for the concentration of LBF has appeared.<sup>14</sup>

## Experimental

**Assay Procedure.**—During the early part of this work, the method of Williams, *et al.*,<sup>4</sup> modified by doubling the vitamins of the basal medium, was used with either *L. bulgaricus* (Gere A) or *Lactobacillus helveticus* 80 as the test organism. After LBF was found to be a bound form of pantothenic acid,<sup>7</sup> a pantothenic acid-free medium with *L. helveticus* 80 was used with superior results. Incubation was for 17 to 24 hours at 37°; other details of this procedure are described elsewhere.<sup>9</sup> A single sample of yeast extract served as an arbitrary standard throughout; it was assigned an activity of one unit per mg. Dry weights were obtained by drying small aliquots containing from 3 to 10 mg. of solids at 100° for 12–18 hours. Material dried in this way is inactive for *L. helveticus*; consequently, the procedure may give an erroneous weight (and hence activity in terms of units per mg.) for fractions of higher purity.<sup>13</sup> As a guide to fractionation, however, the results were fully valid, and because of its convenience this procedure was used throughout.

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