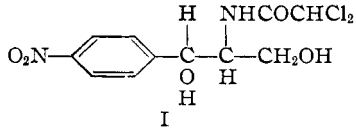
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS & CO.]

Chloramphenicol (Chloromycetin).[1] V. Synthesis

BY JOHN CONTROULIS, MILDRED C. REBSTOCK AND HARRY M. CROOKS, JR.

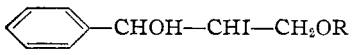
Degradation studies have shown[1a] chloroamphenicol to have the structure I, D-(−)-*threo*-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol



I

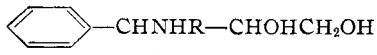
the final proof of structure resting upon synthesis. In this paper we wish to report the synthesis of this structure and the identification of one of the isomeric forms with the antibiotic as produced by fermentation procedures.

Cherbuliez, Neumeier and Lozeron[2] have reported the preparation of compounds related to ephedrine, bearing a hydroxyl group on the terminal carbon of the side-chain and derivatives of this structure by reaction of 2-iodo-1-phenyl-1,3-propandiol, II, its acetate or benzoate, with methylamine. They state that the reaction does



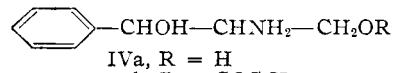
IIa, R = H
b, R = acetyl
c, R = benzoyl

not take place when ammonia is substituted for an alkylamine nor when the halogen is bromine instead of iodine. No proof of the position of the amino group is given. We have found that cinnamyl alcohol bromohydrin, in which the bromine is definitely at position 2, when treated with ammonia yields a small amount of an amine having the empirical formula $C_9H_{13}NO_2$ but more probably the structure IIIa, similar to iso-ephedrine.



IIIa, R = H
b, R = benzoyl

Rabe[3] has shown that iso-ephedrine is formed by the action of methylamine on 1-phenyl-1,2-epoxypropane in addition to ephedrine and *pseudo*-ephedrine. The present case undoubtedly proceeds by the same mechanism. Lott[4] treated IIc, cinnamyl benzoate bromohydrin, with ammonia and isolated a benzoyl derivative, m. p. 165°, to which was assigned structure IVb.



IVa, R = H
b, R = COC_6H_5

We have repeated this preparation and found the product to be neutral, migration from O to N probably taking place during the reaction. The base formed from cinnamyl alcohol bromohydrin and ammonia forms a monobenzoyl derivative m. p. 163° which does not depress the melting point of the Lott benzoyl compound; further, the hydrochlorides of the bases from the two reactions give no depression of mixed melting point. Oxidation of the Lott benzoyl derivative with sodium periodate gives a good yield of formaldehyde and no benzaldehyde indicating structure IIIb. Supportive physical–chemical evidence for assignment of the "iso" structure to this product is given by comparison of the fine structure ultraviolet absorption bands of the amine hydrochloride with those of benzylamine hydrochloride and 2-amino-1-phenyl-1,3-propanediol hydrochloride (*q. v. infra*), Fig. 1. The location and shape of the bands of the 2-amino compound are definitely shifted while those of the base under consideration are almost identical with that of benzylamine.

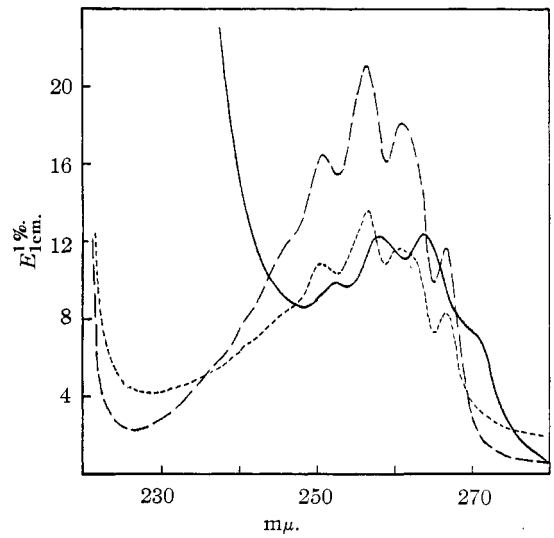


Fig. 1.—Fine structure absorption of 1-phenyl-2-amino-1,3-propanediol, —; benzylamine, — —; and 1-phenyl-1-amino-2,3-propanediol, - - - -; all in 0.1 *N* hydrochloric acid.

Of several alternative potential syntheses of the 2-amino base IVa one of the most attractive utilizes the commercially available aliphatic nitro compounds and is schematically diagrammed in Fig. 2 with no differentiation of stereoisomers.

(1) Chloroamphenicol has been assigned as a generic name for the compound D-*threo*-N-(1,1'-dihydroxy-1-*p*-nitrophenylisopropyl)-dichloroacetamide for which Parke, Davis and Co. has adopted "Chloromycetin" as its trademark.

(1a) Rebstock, Crooks, Controulis and Bartz, THIS JOURNAL, **71**, 2458 (1949).

(2) Cherbuliez, Neumeier and Lozeron, *Helv. Chim. Acta*, **14**, 186 (1931).

(3) Rabe, *Ber.*, **44**, 324 (1911).

(4) Lott, U. S. Patent 2,103,266.

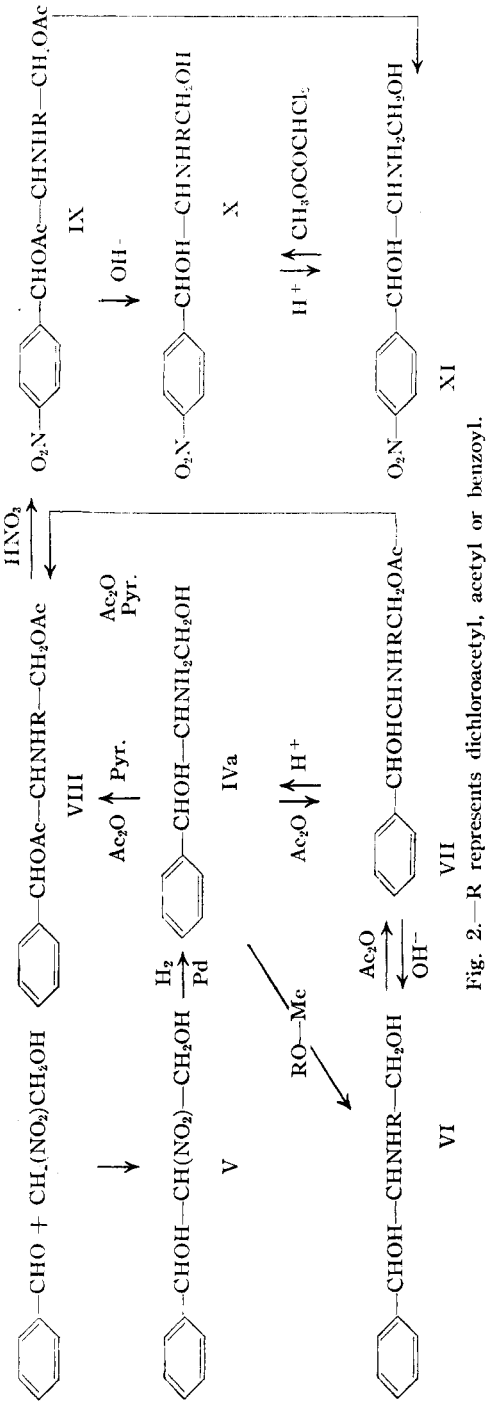
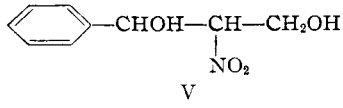


Fig. 2.—R represents dichloroacetyl, acetyl or benzoyl.

It was found that by using Rosenmund's[5] conditions for preparation of 1-phenylnitroethanol, benzaldehyde will condense with β-nitroethanol to form V



V

(5) Rosenmund, *Ber.*, **46**, 1034 (1913).

isolated as its methanol insoluble sodium salt. Catalytic reduction of V in the presence of palladium oxide catalyst gave good yields of IVa.

The base, IVa, 2-amino-1-phenyl-1,3-propanediol, was obtained as a gum, not unexpected in view of its composition, consisting as it does of the racemates of the two stereoisomers. The use of chloroform separated the mixture into a crystalline (A) fraction and an amorphous gum (B). Fraction A was converted to the N-dichloroacetamide derivative, VI, m. p. 158–159° by heating with methyl dichloroacetate. While Oberlin[6] has shown that ephedrine may be nitrated without protection of the amino and hydroxyl groups, a similar procedure applied to A or its dichloroacetamide gave uncrystallized gums; none of the predicted *p*-nitroaminodiol could be isolated. However, when the groups were protected by acylation the resulting 1-phenyl-2-dichloroacetamido-1,3-diacetoxypropane, VIII, m. p. 93–94°, could be nitrated to form a para-nitro derivative, IX, m. p. 134°.

A Kunz[7] hydrolysis of this nitro compound yielded a racemate of a 1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol X, m. p. 171°, corresponding in ultraviolet absorption to chloramphenicol. However, microbiological assay *vs. Shigella paradysenteriae* (Sonnei) showed it to be inactive in the chloramphenicol test. The racemate, therefore, belongs to the *erythro* configuration.

Fraction B upon mild acetylation yielded a crystalline diacetyl derivative VII, m. p. 168–169°. The corresponding diacetyl derivative of the A fraction in contrast melted 110–111°. More vigorous acetylation of the B diacetyl derivative gave a triacetyl derivative VIII, m. p. 79–80° (A series, m. p. 115–116°). Nitration of the triacetyl derivative with fuming nitric acid yielded a para-nitro compound IX, m. p. 146–147° (A series 157–158°). Acid hydrolysis of the nitro-triacetyl derivative of B yielded, after neutralization, 1-*p*-nitrophenyl-2-amino-1,3-propanediol XI, m. p. 141.5–142.5° (A series m. p. 109–110°); the base hydrochlorides melted, A series 180–181°, B series 212–214°. The racemic *p*-nitro base from B was converted to its N-dichloroacetamide X, m. p. 150.5–151.5°, and this was found to have 50% of the activity of natural chloramphenicol when tested *vs. Shigella paradysenteriae*.

The racemic base, XI, from the B or *threo* series was resolved by crystallization of the *d*- and *l*-salts of *d*-camphorsulfonic acid from isopropyl alcohol. The less soluble salt, m. p. 173–174°, $[\alpha]^{27}D$ −1.1° (*c* = 8.45 in water) upon treatment with ammonia gave a (−) base, m. p. 162–163°, $[\alpha]^{27}D$ −23.1° (*c* = 1.58, methyl alcohol); $[\alpha]^{27}D$ −29.3° (*c* = 2.4, 1 *N* hydrochloric acid). The more soluble salt, m. p. 163–164°, $[\alpha]^{27}D$ +25.3° (*c* = 7.35, water), yielded the (+) base, m. p. 162–163°, $[\alpha]^{27}D$ +21.9° (*c* = 1.58, methyl alcohol). The resolved (−) and (+) bases were separately converted to the N-dichloroacetyl derivatives, m. p. 150.5–151.5° each, $[\alpha]^{27}D$ +18.6° (*c* = 4.86 in ethyl alcohol) and $[\alpha]^{27}D$ −18.4° (*c* = 3.3 in ethyl alcohol), respectively. The N-dichloroacetamide from the (−) base was identical in physical and chemical properties with fermentation chloramphenicol and gave 100% inhibition of *Shigella paradysenteriae* (Sonnei) when compared to a fermentation chloramphenicol standard. The corresponding N-dichloroacetamide from the (+) base showed less than ½% chloroamphenicol activity in the same microbiological test.

The N-benzoyl derivatives VI, of the *erythro* (A) and *threo* (B) series bases IVa were prepared for direct comparison with the Lott benzoyl compound. The melting points were, respectively, 157–159° and 165–166°. The mixed melting point of either product with the Lott compound was in the 140–150° range, additional evidence

(6) Oberlin, U. S. Patents 1,856,880, 1,892,532.

(7) Kunz and Hudson, THIS JOURNAL, **48**, 1982 (1926); Wolfrom, Konigsberg and Soltzberg, *ibid.*, **58**, 490 (1936).

that the original assignment of structure IV to the Lott compound was incorrect.

Proof of the assignment of structure VII to the diacyl derivatives was obtained in coöperation with Dr. Loren M. Long. α-Acetamino-β-hydroxypropiophenone[8] was acetylated and the resultant α-acetamino-β-acetoxypropiophenone reduced catalytically with hydrogen and palladium oxide. Crystallization of the reduction product yielded two diacetyl derivatives, m. p. 168–169° (B series) and m. p. 105–107° (A series) which did not depress the melting points of the corresponding diacetyl derivatives obtained by mild acetylation of the bases IVa.

We are indebted to Drs. Leon A. Sweet and Quentin R. Bartz for continued advice and interest in this work, to Dr. John M. Vandenbelt and Misses Geraldine Saladonis and Denise Lundquest for numerous ultraviolet absorption determinations and to Mr. Dwight Joslyn and Mrs. Margaret Galbraith for the many biological assays. We wish to thank Messrs. Arthur Spang and C. E. Childs, Misses Patricia Keller and Renée Schlotterbeck and Mrs. Pat Ramey for the microanalytical data.

Experimental

1-Phenyl-2-nitro-1,3-propanediol.—β-Nitroethanol was first prepared from ethylene iodohydrin and silver nitrite by the method of Demuth and Meyer[9] and purified by their method (distillation, washing free of nitrite ester and redistillation). Later preparations were made from formaldehyde and nitromethane using a 1:10 mole ratio as suggested by Gorsky and Makarov's[10] modification of Henry's[11] method and the product purified by flash-type distillation. During one run, while the excess nitromethane was being removed, *in vacuo*, an explosion occurred which led to our use of the crude mixture on subsequent runs.[12] The technique finally adopted was as follows:

To a stirred suspension of 410 g. of paraformaldehyde in 8.2 liters of commercial nitromethane was added sufficient 3 *N* potassium hydroxide in methanol to initiate reaction, 300 ml. The solution *p*H was about 6.5. After complete solution of the paraformaldehyde and standing an additional one-half hour, 30 ml. of concd. sulfuric acid (35 *N*) was added slowly with vigorous stirring and stirring continued one-half hour after acidification. The reaction was filtered free of potassium sulfate and the filtrate concentrated *in vacuo* at a bath temperature of not more than 35° at 10–15 mm. of mercury pressure. When no more distillate was obtained, 1 liter of methanol was added to the residue and removed *in vacuo* at 35° and 15 mm. (thus removing final amounts of nitromethane by azeotropic distillation). On the basis of Gorsky and Makarov's data the residue (amounting to 1100 g.) consisted of β-nitroethanol approx. 61%, 2-nitro-1,3-propanediol 33% and 2-hydroxymethyl-2-nitro-1,3-propanediol about 3%. Further purification by flash distillation was not necessary since, after condensation with benzaldehyde, only the product from nitroethanol will form an alcohol insoluble sodium salt.

A solution of 550 g. of crude nitroethanol and 600 g. of benzaldehyde in 2300 ml. of methanol was cooled to −10°. To this was added, with stirring, 1400 ml. of cooled (to −10°) methanol containing 130 g. of sodium as sodium methoxide. The temperature rose to 34° and a gel started to appear in three minutes, finally setting to a solid gel. On further stirring for one-half hour the product changed to a powder which was filtered out, washed with dry methanol and dried *in vacuo* at room temperature; yield 830 g. For analysis a sample was crystallized from ice-water by addition of acetone.

Anal. Calcd. for $C_9H_{10}O_4NNa$: N, 6.4; Na, 10.5. Found: N, 6.6; Na, 10.4.

***dl*-1-Phenyl-2-amino-1,3-propanediol.**—A portion of 125 g. of *dl*-1-phenyl-2-nitro-1,3-propanediol sodium salt was dissolved in 450 ml. of glacial acetic acid and hydrogenated at 50 lb. in the presence of 3 g. of palladium oxide. After twenty-three hours 3.15 molecular equivalents of hydrogen had been absorbed. The catalyst was removed and acetic acid distilled at 40° under reduced pressure. The residue was dissolved in 400 ml. of distilled water, extracted twice with ether, and finally made strongly alkaline with concentrated sodium hydroxide. Five extractions with ethyl acetate were required to remove the base effectively. The combined extracts were dried over magnesium sulfate and evaporated. The semicrystalline residue of 69 g. was dissolved in 200 ml. of hot chloroform and kept for seventy-two hours in the refrigerator. A yield of 14.2 g. of crystalline product was collected. The base recrystallized from chloroform melted at 104–105°.[13]

Anal. Calcd. for $C_9H_{13}NO_2$: C, 64.65; H, 7.83; N, 8.38. Found: C, 64.40; H, 7.82; N, 8.41.

Completion of the remaining steps in the synthesis showed the above base to belong to the *erythro* series, A.

For comparison the corresponding base of the *threo* series was prepared. A sample of 12.3 g. of O,N-diacetyl-1-phenyl-2-amino-1,3-propanediol (described below) was hydrolyzed on the steam-bath with 200 ml. of 5% aqueous hydrochloric acid for two and one-half hours. The solvent was removed under reduced pressure and the residue converted to the free base by dissolving in 200 ml. of distilled water, adjusting the *p*H to 11 with concentrated sodium hydroxide and extracting the base with five portions of ethyl acetate. The extracts were dried over magnesium sulfate and evaporated. The residue was purified by three crystallizations from ethyl acetate (m. p. 86–87°), *threo* series, B.

Anal. Found: C, 64.51; H, 7.81; N, 8.37.

***dl*-N,O-Diacetyl-1-phenyl-2-amino-1,3-propanediol.**—The mother liquor from which the crystalline base (m. p. 104–105°) described above had been isolated was evaporated to a gum which consisted of a mixture of the *threo* intermediate together with some *erythro* isomer (not completely separated by the above procedure) and by-products. The residual gum was treated with 100 ml. of acetic anhydride at 70° for fifteen minutes. The acetylating agents were removed *in vacuo* and the semi-crystalline residue recrystallized from 100 ml. of hot absolute ethanol. A yield of 14.5 g. of product melting at 159–165° was obtained. After two recrystallizations from ethanol the acetate melted at 168–169°.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.19; H, 6.82; N, 5.58; O-acetyl, 17.1. Found: C, 62.26; H, 6.78; N, 5.65; O-acetyl, 17.1.

Completion of the synthesis demonstrated that the above diacetate was a member of the *threo* series.

The diacetate of the *erythro* series did not crystallize from the mother liquor but was prepared by acetylating

(8) Long and Troutman, THIS JOURNAL, **71**, 2469 (1949).

(9) Demuth and Meyer, *Ann.*, **256**, 29 (1890).

(10) Gorsky and Makarov, *Ber.*, **67**, 996 (1934).

(11) Henry, *Compt. rend.*, **120**, 1265 (1895).

(12) Critical examination of all factors led to the following conclusions as to the cause of the explosion: (1) The particular lot of commercial nitromethane was quite acidic and a considerable quantity of alkali was necessary to initiate the reaction. (2) Subsequent addition of sulfuric acid was not sufficient to completely react with the metal ion present but only enough to bring the apparent *p*H to about 4–5. Consequently, a considerable quantity of metal ion was present, presumably to some extent as potassium salt of the aci-form of nitromethane which is explosive by virtue of its ease of dehydration to potassium fulminate (*cf.* Sidgwick, "Organic Compounds of Nitrogen," 1937, p. 236). While no difficulties were encountered in the distillation of β-nitroethanol prepared by the ethylene-iodohydrin method it is possible that the more highly substituted nitromethanes of the second procedure may be less stable and hence render distillation hazardous. We feel that such an operation should be carried out behind an adequate (shatterproof) shield as a matter of extra caution even though our inclination is that the metal salt of the aci-form of nitromethane is the inherent source of difficulty.

(13) Melting points were determined on a calibrated Fisher-Johns block.

1 g. of base (m. p. 104°) with 3 ml. of acetic anhydride for thirty minutes at 100°. The product was recrystallized twice from ethyl acetate (m. p. 110–111°). Found: C, 62.39; H, 6.61; N, 5.68; O-acetyl, 17.3.

A Kunz[7] hydrolysis of the *threo* diacetate indicated one acetyl was bound in ester and the other in amide linkage. The N-acetyl derivative was isolated from a neutralized Kunz hydrolysate by five extractions with ethyl acetate. The combined extracts were dried over magnesium sulfate and evaporated. The product recrystallized from ethyl acetate melted at 136–137°, second form, m. p. 144–145°.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.70. Found: C, 63.21; H, 7.23; N, 6.55.

The corresponding monoacetyl derivative of the *erythro* base (m. p. 103–104°) was prepared by treating 1 g. of base with 3 ml. of acetic anhydride at room temperature. The reaction mixture warmed spontaneously and the product crystallized. Excess acetylating reagent was removed *in vacuo* and the product recrystallized from ethyl acetate (m. p. 106.5–107°). Found: C, 63.33; H, 7.25.

N-Dichloroacetamide, *erythro* Series.—A 1.7-g. sample of the total amorphous amino-diol from the palladium reduction was heated one and one-quarter hours with an equal weight of methyl dichloroacetate. The solid which appeared was washed with petroleum ether and recrystallized from water, 0.35 g., m. p. 158–159°.

Anal. Calcd. for $C_{11}H_{13}NO_3Cl_2$: C, 47.5; H, 4.7; N, 5.0. Found: C, 47.2; H, 5.1; N, 5.3.

***dl*-Triacetyl-1-phenyl-2-amino-1,3-propanediol.**—Evaporation of the mother liquor from the isolation of the active series diacetyl derivative yielded a gum which was further acetylated by refluxing with 100 ml. of acetic anhydride for two hours. The acetylating reagent was removed *in vacuo* and the dark brown tar dissolved in 150 ml. of absolute ethanol to which an equal volume of low petroleum ether was added. After standing overnight in the refrigerator a crop of gummy crystals was collected. One recrystallization from absolute ethanol yielded 10.3 g. (m. p. 113–115°). The recrystallized triacetate melted at 115–116°.

Anal. Calcd. for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.40; H, 6.54; N, 4.73.

Further steps in the synthesis showed this intermediate to belong to the *erythro* series. The same product was obtained by acetylating N-acetyl or N,O-diacetyl-1-phenyl-2-amino-1,3-propanediol with pyridine–acetic anhydride.

No triacetate of the *threo* series could be induced to crystallize from the residue although further working of the gum (see below) showed that some active intermediate was present. The *threo* triacetate was prepared from the 168–169° diacetate described above. Seven grams of *dl*-*threo*-N,O-diacetyl-1-phenyl-2-amino-1,3-propanediol were treated with 10 ml. of pyridine and 12 ml. of acetic anhydride at room temperature for forty-four hours. The acetylating reagents were removed and the residue recrystallized from 25 ml. of ethyl acetate to which four volumes of low petroleum ether was gradually added. After standing overnight 7.1 g. of crystalline triacetate was obtained. A sample twice recrystallized from ethanol–low petroleum ether melted at 79–80°. Found: C, 61.39; H, 6.70; N, 4.90.

N-Dichloroacetamide, N,O-Diacetyl *erythro* Series.—A solution of 1.0 g. of N-dichloroacetamide, m. p. 158–159°, in 5 ml. each of pyridine and acetic anhydride was allowed to stand overnight. After quenching in ice-water the precipitated oil was taken up in ether and washed with dilute hydrochloric acid, dilute sodium carbonate solution and water. Evaporation of the ether yielded 0.9 g., m. p. 92–94°, recrystallized from dilute methanol, m. p. 94°. Mol. wt. (from ultraviolet absorption) 346; calcd. for $C_{15}H_{17}O_5NCl_2$, 362.

***dl*-Triacetyl-1-*p*-nitrophenyl-2-amino-1,3-propanediol.**—Fifteen grams of *dl*-triacetyl-1-phenyl-2-amino-1,3-propanediol (*threo* series) was added in portions during ten minutes to 40 ml. of fuming nitric acid (sp. gr., 1.5) which had been decolorized by addition of sulfamic acid. The reaction temperature was kept at 30–35° by adding Dry Ice directly to the reaction mixture. The nitration mixture was allowed to stand for fifteen minutes longer at room temperature, then quenched on ice, and immediately neutralized with solid sodium bicarbonate. The product separated as a gum. The mixture was extracted three times with ethyl acetate and the combined extracts washed with one volume of water, dried, and evaporated at reduced pressure. The residue of 17.9 g. crystallized upon standing overnight. The crude product was hydrolyzed without further purification.

The pure para isomer was isolated by recrystallizing the crude nitration triacetate intermediate from another run successively from acetone–low petroleum ether, twice from ethanol (m. p. 140–143°) and finally twice from water (m. p. 146–147°). It was not profitable in practice to separate the para isomer at this point since the recrystallizations did not represent quantitative recovery of the desired product.

Anal. Calcd. for $C_{15}H_{18}N_2O_7$: C, 53.25; H, 5.36; N, 8.28; O-acetyl, 25.5. Found: C, 53.02; H, 5.45; N, 8.44; O-acetyl, 25.7.

Nitration with mixed acid was also found to proceed satisfactorily. Fifteen grams of the *erythro* series triacetate (m. p. 115–116°) was added in portions to 50 ml. of 1:1 nitric acid (sp. gr. 1.42), sulfuric acid (sp. gr. 1.84) at −20°. After thirty minutes the reaction mixture was transferred to an ice-bath for fifteen minutes, then kept at room temperature for twenty minutes longer. The nitration mixture was quenched on ice and the crystalline product which separated was filtered and washed well with ice water. The triacetate was recrystallized twice from hot absolute ethanol, once from ethyl acetate, m. p. 152–153°, and finally twice more from ethanol, m. p. 157–158°. Found: C, 53.55; H, 5.53; N, 8.18; O-acetyl, 25.9.

***dl*-1-*p*-Nitrophenyl-2-dichloroacetamido-1,3-diacetoxypropane, *erythro* Series.**—One quarter milliliter each of concd. nitric and sulfuric acids were mixed and cooled to −30°. To the mixture was added 200 mg. of *dl*-*erythro*-1-phenyl-2-dichloroacetamido-1,3-diacetoxypropane in portions. Solution occurred as the temperature was allowed to rise to 0°. After five minutes at room temperature the solution was quenched with ice, and extracted with ethyl acetate. Evaporation of the washed and dried extract yielded a crystalline solid which was washed with ether and finally recrystallized from ethanol, m. p. 134°. A mixed melting point with diacetyl chloramphenicol (141–142°) was 116°. Ultraviolet absorption indicated a para nitro compound.

Anal. Calcd. for $C_{15}H_{16}O_7N_2Cl_2$: C, 44.25; H, 3.93. Found: C, 43.65; H, 3.86.

***dl*-N-Acetyl-1-*p*-nitrophenyl-2-amino-1,3-propanediol.**—A sample of 840.7 mg. of *dl*-*erythro*-triacetyl-1-*p*-nitrophenyl-2-amino-1,3-propanediol (m. p. 157–158°) was hydrolyzed with 100 ml. of 0.1 *N* sodium hydroxide under Kunz conditions. The neutralized reaction mixture was extracted five times with ethyl acetate. The N-acetyl derivative was obtained by evaporating the dried extracts and purified by two recrystallizations from ethanol (m. p. 195–196°).

Anal. Calcd. for $C_{11}H_{14}N_2O_5$: C, 51.97; H, 5.52; N, 11.03. Found: C, 52.15; H, 5.49; N, 11.23.

The N-acetyl derivative of the *threo* series was prepared in the same manner (m. p. 166.5–167.5°). Found: C, 52.22; H, 5.87; N, 11.26.

***dl*-*p*-Nitrophenyl-2-amino-1,3-propanediol.**—The crude residue obtained by evaporation of the ethyl acetate extract of the nitrated triacetyl *threo* intermediate (17.0 g.) was hydrolyzed for two and one-half hours with 200 ml. of 5% hydrochloric acid on the steam-bath. The hydrolysate was extracted twice with ether and the aqueous solution of the hydrochloride taken to dryness under

reduced pressure. The crude hydrochloride (11.4 g.) was dissolved in 30 ml. of ice-cold water and the *p*H adjusted to 11 with concentrated sodium hydroxide solution, whereupon the free base crystallized. After standing overnight in the refrigerator 6.1 g. of product was collected (m. p. 131–133°). The base was recrystallized once again from 35 ml. of hot water to a yield of 4.6 g. (m. p. 139–141°). A final recrystallization yielded the pure para isomer (m. p. 141.5–142.5°).

Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.69; N, 13.20. Found: C, 51.03; H, 5.92; N, 13.30.

The corresponding nitro base of the *erythro* series was prepared in the same manner as above except that in this case the free base failed to crystallize from the alkaline solution even though the hydrochloride was purified by recrystallization before attempting the conversion. It was necessary to extract the base with five portions of ethyl acetate. The combined extracts were dried and evaporated. At this point the base crystallized readily. The product was recrystallized twice from isopropyl alcohol–ethylene dichloride (1:3), (m. p. 109–110°). Found: C, 51.09; H, 5.92; N, 13.15.

The hydrochlorides of both bases were purified by three recrystallizations from 95% ethanol.

Anal. Calcd. for $C_9H_{13}N_2O_4Cl$: C, 43.47; H, 5.27; N, 11.27. Found: C, 43.42; H, 5.24; N, 11.23 (*erythro* series, m. p. 212–214°). C, 43.72; H, 5.43; N, 11.33 (*threo* series, m. p. 180–181°).

A 300-mg. portion of *dl-erythro*-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-diacetoxypropane was hydrolyzed in 25 ml. of 6 *N* hydrochloric acid for one and three-quarter hours on the steam-bath. After concentrating *in vacuo* the base was extracted at *p*H 11 with ethyl acetate and the hydrochloride precipitated from the dried ethyl acetate solution by addition of ethanolic hydrogen chloride. After three recrystallizations from *n*-propanol the product melted 216–218°, no depression on mixing with the *erythro* hydrochloride from the triacetyl compound above.

***dl*-N-Dichloroacetyl-1-*p*-nitrophenyl-2-amino-1,3-propanediol.**—The N-dichloroacetamide was prepared by heating 350 mg. of the *dl-threo* isomer of the corresponding nitro base with 2.5 ml. of methyl dichloroacetate on the steam-bath for one and one-half hours. The mixture was extracted with three 15-ml. portions of low petroleum ether to remove excess ester. The residue was crystallized twice from ethyl acetate–low petroleum ether and finally twice from water (m. p. 150.5–151.5°). One mg./ml. was equivalent to 500 γ/ml. natural chloramphenicol (inhibition of *S. paradysenteriae*).

Anal. Calcd. for $C_{11}H_{12}N_2O_5Cl_2$: C, 40.88; H, 3.74; N, 8.67. Found: C, 41.17; H, 3.82; N, 8.81.

The corresponding amide of the *erythro* series was prepared in the same manner. The derivative was recrystallized from ethanol–low petroleum ether twice and finally water (m. p. 172.5–173.5°). One mg./ml. was equivalent to <1 γ/ml. natural chloramphenicol (inhibition of *S. paradysenteriae*). Found: C, 40.96; H, 4.07; N, 8.44.

The N-dichloroacetyl acetyl derivative, *erythro* series, was also obtained by Kunz hydrolysis of 500 mg. of the 134° dichloroacetamido-diacetoxy compound above, recrystallized from water and from ethylene dichloride m. p. 171°. The ultraviolet absorption curve was essentially identical with that of chloramphenicol but bioassay showed less than 0.5% chloramphenicol activity.

Anal. Found: C, 41.17; H, 3.88.

Resolution of *dl*-*p*-Nitrophenyl-2-amino-1,3-propanediol, B (*threo*) Series.—Ten grams of *dl*-1-*p*-nitrophenyl-2-amino-1,3-propanediol (m. p. 140–141°) and 10.95 g. of *d*-camphorsulfonic acid (Eastman Kodak Co.) were dissolved in 400 ml. of isopropyl alcohol by heating on the steam-bath for ten minutes. After standing overnight at room temperature 5.4 g. of salt (m. p. 166–168°) was obtained. Recrystallization from 135 ml. of isopropyl alcohol yielded 3.59 g. (m. p. 170–172°). A final recrystallization yielded the pure *ld*-salt (m. p. 173–174°) $[\alpha]^{27}D$ −1.1° (*c* = 8.45% in water).

Anal. Calcd. for $C_{19}H_{28}N_2O_8S$: C, 51.34; H, 6.35; N, 6.30. Found: C, 51.61; H, 6.28; N, 6.39.

The mother liquor from the first crop of 5.4 g. of crude *ld*-salt (m. p. 166–168°) was diluted with two volumes of ether. A crop of 4.68 g. (m. p. 155–158°) was recrystallized once again from 94 ml. of isopropyl alcohol to a yield of 3.0 g. (m. p. 163–164°) pure *dd*-salt; $[\alpha]^{27}D$ +25.3 (*c* = 7.35% in water). Found: C, 51.41; H, 6.47; N, 6.42.

A second crop of *ld*-salt was obtained by evaporating the mother liquor from the 4.68 g. of *dd*-salt to 250 ml. and allowing to stand for a week. A yield of 1.55 g. (m. p. 165–170°) purified by an additional crystallization to 1.2 g. (m. p. 172–174°) was obtained.

Evaporation of the mother liquor from the 1.55 g. to 130 ml. and dilution with two volumes of ether then yielded a second crop of *dd*-salt which was recrystallized as above to a yield of 0.47 g. (m. p. 163–164°) of pure *dd*-salt.

By concentrating the mother liquor, another crop of *ld* was isolated and from that filtrate by dilution with ether, another crop of *dd*. This process of alternate concentration to obtain *ld* and ether precipitation to obtain *dd*-salt was repeated until most of the base had been resolved.

***l* and *d*-*p*-Nitrophenyl-2-amino-1,3-propanediol B (*threo*) Series.**—A sample of 1.60 g. of the *d*-camphorsulfonate of the *l*-base was dissolved in 20 ml. of distilled water and the *p*H adjusted to 10 with ammonium hydroxide. The precipitated base was filtered after twenty minutes and recrystallized from 30 ml. of hot water to a yield of 670 mg. (m. p. 162–163°). The free base did not depress the melting point of the base obtained by acid or alkaline degradation of natural chloramphenicol; $[\alpha]^{27}D$ −23.1° (*c* = 1.58%, in methanol); $[\alpha]^{27.5}D$ 29.3° (*c* = 2.4% in 1.0 *N* hydrochloric acid).

Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.69; N, 13.20. Found: C, 50.80; H, 5.90; N, 13.27.

The *d*-base, m. p. 162–163°, was prepared from the corresponding *d*-camphorsulfonate; $[\alpha]^{27}D$ +21.9° (*c* = 1.58%, in methanol). Found: C, 50.98; H, 5.99; N, 13.49.

***l* and *d*-N-Dichloroacetyl-1-*p*-nitrophenyl-2-amino-1,3-propanediol B (*threo*) Series.**—The resolved bases were converted to *d*- or *l*-N-dichloroacetyl derivatives by the procedure described for the corresponding *dl*-compound. The crude amides were recrystallized from ethylene dichloride, ethyl acetate–low petroleum ether, and water.

The *l*-base yielded a product identical with naturally occurring chloramphenicol (m. p. 150.5–151.5°); $[\alpha]^{27}D$ +18.6° (*c* = 4.86%, in ethanol), 1 γ/ml. ≈ 1 γ/ml. chloramphenicol (inhibition of *S. paradysenteriae* (Sonnei)).

Anal. Calcd. for $C_{11}H_{12}N_2O_5Cl_2$: C, 40.88; H, 3.74; N, 8.67. Found: C, 41.14; H, 3.84; N, 8.25.

The *d*-base yielded the enantiomorph (m. p. 150.5–151.5°); $[\alpha]^{27}D$ −18.4° (*c* = 3.3%, in ethanol). Inactive against *S.* Sonnei. (1 mg./ml. ≈ <1 γ/ml. chloramphenicol). Found: C, 41.26; H, 4.02; N, 8.81.

Assignment of Positions 2 and 3 to Diacetyl Derivatives VII.—Fifteen grams of α-acetamino-β-hydroxypropiophenone furnished by Dr. Long[8] was dissolved in 15 ml. of pyridine and 15 ml. of acetic anhydride. After standing overnight the solvents were removed and the crystalline residue recrystallized from 50 ml. of ethyl acetate; 6.17 g. of product was obtained. An additional 3.5 g. was obtained by taking the mother liquor to dryness and dissolving in 100 ml. of ether. The total product was recrystallized from ether (m. p. 84–85°).

Anal. Calcd. for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.63. Found: C, 62.88; H, 6.29; N, 5.76.

Eight and one-half grams of α-acetamino-β-acetoxypropiophenone was hydrogenated in the presence of 2.0 g. of palladium oxide using 150 ml. of ethanol as the solvent at 50° for three hours. The catalyst was filtered off and the solvents removed under reduced pressure. The

crystalline residue was recrystallized from 40 ml. of hot absolute ethanol. A yield of 3.0 g. (first and second crops; m. p. 159–161°) was obtained. Recrystallization from ethanol yielded the 168–169°) diacetate of the *threo* (B) series. The ethanol mother liquor was concentrated to 10 ml. and 30 ml. of ether added. After standing overnight in the refrigerator, 2.86 g. was obtained (m. p. 105–107°); no depression of diacetate of the *erythro* (A) series. Evaporation of this mother liquor to dryness and crystallization of the residue from 15 ml. of ethylene dichloride yielded 1.60 g., m. p. 103–106°, of additional crude *erythro* diacetate.

1-Phenyl-1-benzamido-2,3-propanediol.—(Method of Lott[4].) To a stirred suspension of 23 g. of yellow mercuric oxide in 220 ml. of moist ether containing 25 g. of cinnamyl benzoate was added 18.5 g. of bromine over a period of thirty-five minutes at the reflux temperature of the ether (maintained by illumination with a "photoflood" bulb). After comp etion of the bromine addition and an additional twenty minutes of stirring the suspension was filtered and the ether washed with 20% aqueous potassium iodide solution and with 30 ml. of 5% sodium bisulfite solution. The dried ether solution was evaporated *in vacuo* until the dark yellow residual oil was free of ether. The oil was transferred to a stainless steel bomb with 50 ml. of 15% ethanolic ammonia and the bomb heated six hours at 100°. The bomb contents were filtered to remove a brown precipitate and the filtrate concentrated *in vacuo*. The residue was taken up with ether and water, silky needles being slowly deposited from the ether phase: 5.4 g., m. p. 154–158°. After recrystallization from aqueous methanol the product melted 164–165°.

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.85; H, 6.33; N, 5.17. Found: C, 71.30; H, 6.50; N, 5.09, 5.14.

A 1-g. sample of the benzoyl compound was hydrolyzed with 6 *N* hydrochloric acid to give a hydrochloride m. p. 222–224° after crystallization from *n*-propanol, mixed m. p. with hydrochloride (216–217°) below, 220–222°.

1-Phenyl-1-amino-2,3-propanediol.—A 13.4-g. sample of cinnamyl alcohol was dissolved in 100 ml. of ethanol and added to 200 ml. of 50% ethanol, with stirring, simultaneously with 16 g. of bromine in 500 ml. of water. After removal of some tarry material by filtration the filtrate was concentrated *in vacuo* and the residue extracted with three equal volumes of ether. Evaporation of the dried ether extracts left a bromine-containing pale yellow oil. Titration of two samples of the oil with sodium periodate showed no oxidation occurred and the position of the bromine was therefore C-2 of the propane chain. Six grams of the 1-phenyl-2-bromo-1,3-propanediol with 15 ml. of saturated ethanolic ammonia and 100 ml. of 28% aqueous ammonia was heated sixteen hours at 85–95° in a stainless steel bomb. The bomb contents were filtered and then extracted with three equal portions of ethyl acetate. The dried ethyl acetate solution was concentrated to a brownish oil and this treated with methanolic hydrogen chloride and ether. The gummy solid precipitate was recrystallized from *n*-propanol, m. p. 216–217°, and amounted to 110 mg.

Anal. Calcd. for $C_9H_{14}NO_2Cl$: C, 53.07; H, 6.88. Found: C, 53.72; H, 7.31.

A 15-mg. sample of the hydrochloride was treated with two micro-drops of benzoyl chloride in excess 1 *N* sodium hydroxide. The precipitate was recrystallized from methanol and from water, m. p. 163°. This did not depress the melting point of a sample of the monobenzoate prepared by the Lott method (see above), m. p. 165°.

A 33.6-mg. sample of the Lott benzoyl derivative was treated with 5 ml. of 0.13 *M* sodium periodate at 27° for one and one-half hours. The solid appeared to dissolve with reprecipitation of another solid. The solution was then extracted with ether, the solid suspending in the ether phase. The aqueous phase was treated with 1 ml. of 6.5% sodium arsenite solution and 4 drops of 20% potassium iodide solution for ten minutes. Addition of one drop of acetic acid and five drops of dimedon solution gave a copious precipitate which was collected, m. p. 186°, m. m. p. with authentic formaldehyde dimedon, no depression. Evaporation of the ether phase left a solid residue having no odor of benzaldehyde. After washing with water the solid melted 115–118°, m. m. p. with starting material (165°) was 105–109°.

1-Phenyl-2-benzamido-1,3-propanediol.—A 1-g. sample of the B series (*threo*) base IVa was treated with benzoyl chloride and 1 *N* sodium hydroxide with shaking. After crystallization from acetone the product melted 145–146°. The corresponding A derivative melted 165–166°. Analysis showed these to be dibenzoyl compounds.

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.85; H, 6.33. Calcd. for $C_{23}H_{21}NO_4$: C, 73.5; H, 5.6. Found: A: C, 73.23; H, 5.5; B: C, 73.4; H, 6.0.

The two dibenzoyl derivatives, 150 mg. each, were separately hydrolyzed with 1.2 equivalents of sodium hydroxide in 30 ml. of 50% methanol at reflux for one hour. Recrystallization from ethyl acetate yielded the monobenzoyl derivatives, A series m. p. 157–159°, B series m. p. 165–166°. Mixed melting points with the Lott benzoyl compound were, A, m. p. 142–149°, B, 140–147°.

Anal. Found: A: C, 70.76; H, 6.43. B: C, 70.69; H, 6.39.

Summary

The structure previously assigned to chloramphenicol has been synthesized and one of the isomers found to correspond in all properties to the fermentation-produced antibiotic.

This is the first practical synthesis of a major antibiotic.

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