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Chloramphenicol (Chloromycetin).[1] IV.[1a] Chemical Studies

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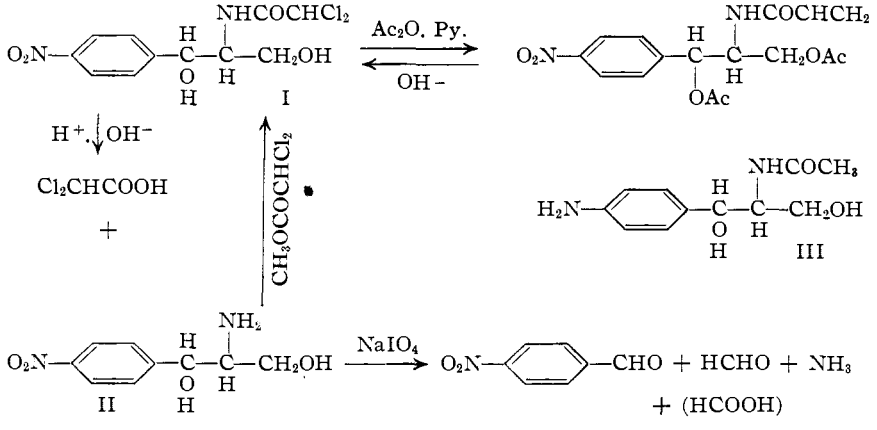
The discovery and isolation of chloramphenicol has been described in previous papers from this Laboratory[1a] and from the University of Illinois.[2] The present paper reports the studies which have led to the assignment of formula I as the structure. The proof of this structure by synthesis is reported in the next paper.



Chloramphenicol is a relatively stable, neutral compound having a sharp melting point (150.1°), soluble in many organic solvents, sparingly soluble in water and capable of sublimation in high vacuum without decomposition. It is optically active: $[\alpha]^{25}D$ + 19° in ethanol and −25.5° in ethyl acetate. It contains carbon, hydrogen, non-ionic chlorine and does not contain sulfur. The molecular weight in camphor (micro-Rast) is about 310. The empirical formula which best satisfies the analytical data is $C_{11}H_{12}Cl_2N_2O_5$.

Probably the most characteristic physical property of the compound is its absorption spectrum in the ultraviolet: $E^{1\%}_{1\,cm.}$ 298 at 278 mμ, Fig. 1. Because of the extensive studies on the relation of ultraviolet absorption to chemical structure, particularly in the benzene series, by Mr. Leonard Doub and Dr. John M. Vandenbelt[3] of these laboratories, the curve of chloramphenicol was referred to these workers for their interpretation. The position and shape of the absorption maximum suggested a nitrobenzene derivative (Fig. 1). This postulation was accepted with some reservation since no natural product has heretofore been reported containing a nitro group. After the presence of the nitro group had been demonstrated chemically the ultraviolet data further indicated that the chlorine was probably not in the benzene ring (supported by the data on the reduction product, Fig. 2) but that, at most, an alkyl group was *para* to the nitro substituent.

The chemical characterization of chloramphenicol gave the following data: The chlorine is not affected by cold or boiling 3% alcoholic silver nitrate solution. Absence of carbonyl function is indicated by lack of evidence of derivative formation with thiosemicarbazide or other carbonyl reagent. The compound reduces neutral permanganate solution slowly, alkaline permanganate rapidly. The biuret test is not characteristically positive but green crystals of a copper salt appear on standing. The formation of ephedrine and propadrine cuprous oxide salts under similar conditions has been described by Chen and Kao[4] who refer to Nagai as discoverer of the reaction. In view of the structural relationship of the base which would be liberated under the conditions of the test (see the alkaline degradation experiment described below) it is likely that the above product is a similar type of salt. Catalytic reduction (Adams platinic oxide) indicates rapid absorption of six molecular equivalents of hydrogen and slower absorption of two additional equivalents. The resulting solution shows ionic halogen. Similar hydrogenation in the presence of palladium oxide shows absorption of five molecular equivalents of hydrogen in an hour. The resulting solution shows ionic halogen, the presence of a base and absorption in the ultraviolet similar to that of *p*-toluidine, Fig. 2. The base is presumably compound III although no product was isolated in this instance.

The nature of the nitrogen atoms was determined by the following experiments. No primary amino group can be detected by a Van Slyke determination on the antibiotic itself but if a solution of chloramphenicol be allowed to stand overnight with 0.1 *N* sodium hydroxide solution

(1) Chloramphenicol 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) Previous (unnumbered) papers on this subject from this Laboratory: (a) Ehrlich, Bartz, Smith, Joslyn and Burkholder, *Science*, **106**, 417 (1947); (b) Smith, Joslyn, Gruhzit, McLean, Penner and Ehrlich, *J. Bact.*, **55**, 425 (1948); (c) Bartz, *J. Biol. Chem.*, **172**, 445 (1948).

(2) Carter, Gottlieb and Anderson, *Science*, **107**, 113 (1948); Gottlieb, Bhattacharyya, Anderson and Carter, *J. Bact.*, **55**, 409 (1948).

(3) Doub and Vandenbelt, THIS JOURNAL, **69**, 2714 (1947).

(4) Chen and Kao, *J. Am. Pharm. Assn.*, **15**, 625 (1926), cite Nagai, *J. Pharm. Soc. Japan* (1892).

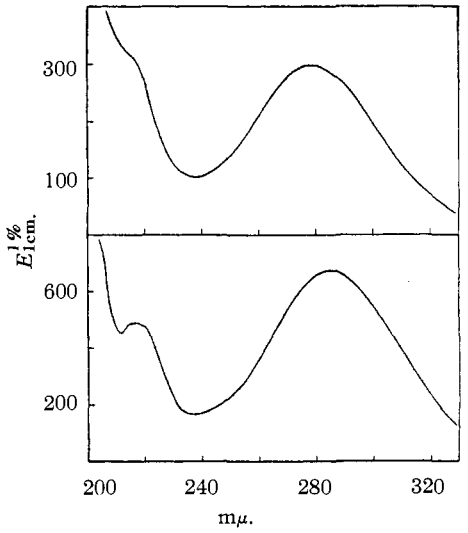


Fig. 1.—Top, chloramphenicol in water; bottom, *p*-nitrotoluene in water (diluted from methanol solution).

the resulting solution gives analytical figures for one primary amino group.[4a] A test with ferrous hydroxide[5] on a solution of chloramphenicol is positive for nitrogen in an oxidized state. The presence of a nitro group is indicated by the fact that reduction with zinc and calcium chloride solution followed by treatment with benzoyl chloride and sodium acetate and finally treatment with ferric chloride and sulfuric acid gives a positive test.[6] An oxidation state higher than that of an hydroxylamine is indicated by a negative test when the zinc reduction is omitted. Reduction of chloramphenicol with tin and hydrochloric acid followed by diazotization and coupling with β-naphthol gives a heavy orange-red precipitate. The tin reduced material shows ultraviolet absorption essentially that of the palladium reduced product and similar to *p*-toluidine.

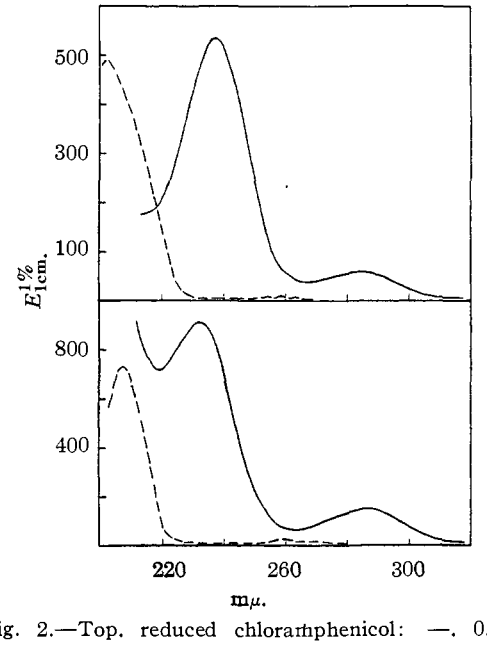


Fig. 2.—Top, reduced chloramphenicol: —, 0.1 *N* sodium hydroxide; — —, 0.1 *N* hydrochloric acid. Bottom, *p*-toluidine: —, *p*H 11; — —, 0.5 *N* hydrochloric acid.

Chloramphenicol, treated with acetic anhydride in pyridine, gives a diacetyl derivative. Hydrolysis of this derivative by the method of Kunz[7] demonstrates that this is a di-O-acetyl compound and chloramphenicol is recoverable from the hydrolysate.

Hydrolysis of chloramphenicol with either acid or alkali yields a volatile acid and a crystalline, optically active base II. The volatile acid was identified as dichloroacetic acid (characterized by its S-benzylthiouronium salt). The base readily forms a crystalline monohydrochloride. Analytical data on both the base and its hydrochloride as well as the intensity of the nitro peak in the ultraviolet, Fig. 3, indicates an empirical formula of $C_9H_{12}N_2O_4$ for the base. The base was further characterized by preparation of an N-*p*-nitrobenzoyl, an N-acetyl and an N,O,O-triacetyl derivative. Treatment of the base with methyl dichloroacetate gives a dichloroacetamide which is identical with natural chloramphenicol.

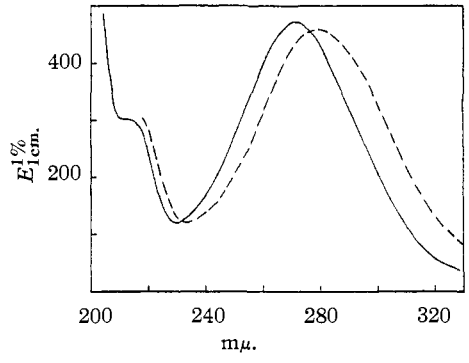


Fig. 3.—Base from chloramphenicol in 0.1 *N* HCl (—) and in 0.1 *N* NaOH (— —).

Chloramphenicol was not affected by periodic acid under the conditions ordinarily employed for determination of vicinal hydroxyl groups. However, when the base which is derived by hydrolysis was so treated it was found to consume two equivalents of the reagent and the products found were one molecular equivalent each of ammonia, formaldehyde and *p*-nitrobenzaldehyde. Quantitative data were not obtained for formic acid

(4a) We are indebted to Dr. Gertrude Rodney for these determinations.

(5) Shriner and Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., p. 74.

(6) Schneider, "Qualitative Organic Microanalysis," p. 195–198.

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

which must represent the remaining carbon atom of the base. These reactions place the amino group at position 2 of the propyl chain.

The conclusions of structure based on the ultraviolet absorption were thus completely confirmed.

For stereochemical purposes the antibiotic may be considered a member of the ephedra series. The stability of the base under acid and alkaline hydrolysis and under various acylation conditions as well as the amount of rotation of the base and its derivatives indicates by analogy that it is sterically classified in the more stable *pseudo*-ephedrine series. Further, the sign of optical rotation of the antibiotic, the derived base, its hydrochloride and *p*-nitrobenzamide indicate that it may be considered a derivative of a substituted (*l*)-nor-*pseudo*-ephedrine (Table I).

TABLE I

	l-nor ephedrine[8]		*l-nor-ψ-* ephedrine[8]		Base from chloramphenicol	
	M. p., °C.	$[\alpha]^{27}D$	M. p., °C.	$[\alpha]^{27}D$	M. p., °C.	$[\alpha]^{25}D$
Base	50	−14.56	77–78	−32.64	162.3	−23.1
Base HCl	171–172	−33.27	180–181	−42.68	210–211	−26.9
Base *p*-NO_2-benzamide	175–176	−49.58	199	−105.13	204–205	−140.2

On the basis of these data the most probable structure for chloramphenicol (Chloromycetin) is D-(−)*threo*-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol[9] (N-(1,1′-dihydroxy-1-*p*-nitrophenylisopropyl)-dichloroacetamide).

Chloramphenicol is, so far as we know, the first naturally occurring compound which contains a nitro group or which is a derivative of dichloroacetic acid.

The relative *in vitro* antibacterial action of some derivatives of the chloramphenicol base is given in Table II: test organism, *Shigella paradysenteriae* (Sonnei); assay type, turbidimetric, 50% inhibition; standard, natural chloramphenicol.

TABLE II

	% Activity
Reconstituted chloramphenicol	100
Free base from chloramphenicol	1.8
N-Acetyl base	14.3
N-Ethylcarbamate	3.0
Phenyl thiourea	4.0
p-Nitrobenzamide	<6.0
Reduced base	<0.1
Reduced N-acetyl base	0.1

We wish to acknowledge our indebtedness to Mr. Leonard Doub and Dr. John M. Vandenbelt for invaluable aid in interpretation of ultraviolet absorption data, to Mr. Dwight Joslyn and Mrs. Margaret Galbraith for numerous bio-assays and to Dr. Leon A. Sweet for enthusiastic advice and encouragement. We wish to thank Mr. A. W. Spang and Miss Patricia Keller for the many microanalyses.

(8) Data from Nagai and Kanao, *Ann.*, **470**, 157 (1929).

(9) The configuration of the asymmetric carbon atoms of the ephedrine series has been related to mandelic acid and to alanine by Freudenberg, THIS JOURNAL, **54**, 234 (1932), and *Ann.*, **510**, 223 (1934). We have arrived at this designation through consideration of chloramphenicol as a substituted glycerol and use of the ephedrine series relationships as established by Freudenberg.

Experimental

Degradation of Chloramphenicol with 1.0 *N* Hydrochloric Acid.—A 5-g. sample of chloramphenicol was heated on the steam-bath for two hours with 75 ml. of 1.0 *N* hydrochloric acid. The reaction mixture was cooled and extracted four times with equal portions of acid-free ether. Evaporation of the ether extracts yielded dichloroacetic acid (see below for details of identification). The aqueous residue was taken to dryness at 45° and reduced pressure. A crystalline hydrochloride was obtained (3.4 g. yield) which had a negative rotation $[\alpha]^{26.5}D$ −26.9° (*c* = 4.0%, in methanol). Recrystallization from 50 ml. of absolute ethanol yielded 1.73 g. of product (m. p. 210–211°[10]); $[\alpha]^{26.8}D$ −26.8° (*c* = 4.16%, in methanol). By diluting the mother liquor with an equal volume of low petroleum ether a second crop of 1.18 g., m. p. 209–210°; $[\alpha]^{26.8}D$ −26.9° (*c* = 4.65%, in methanol) was obtained. The product was finally recrystallized from *n*-propanol.

Anal. Calcd. for $C_9H_{13}O_4N_2Cl$: C, 43.47; H, 5.27; N, 11.27. Found: C, 43.74; H, 5.41; N, 11.13.

The hydrochloride could be converted to the free base by the following procedure. The *p*H of an ice-cold solution of 1.93 g. of the hydrochloride in 10 ml. of distilled water was adjusted to 9–10 with 0.5 *N* sodium hydroxide. During addition of alkali the free base crystallized. The product was filtered, washed with ice-water, and recrystallized from hot water. The free base (yield, 1.39 g.) melted at 160–162° and did not depress the melting point of base obtained by the alkaline degradation procedure described below $[\alpha]^{25.8}D$ −22.8° (*c* = 3.0%, in methanol). The starting hydrochloride could be obtained by evaporation of a dilute aqueous hydrochloric acid solution of the base; $[\alpha]^{25.8}D$ −26.5° (*c* 4.58%, in methanol).

Degradation of Chloramphenicol with 0.1 *N* Sodium Hydroxide.—Treatment of 3 g. of chloramphenicol with 200 ml. of 0.1 *N* aqueous sodium hydroxide in an amber bottle at room temperature for eighteen hours resulted in 99.5% inactivation of the antibiotic when assayed against *Shigella sonnei*. The alkaline solution was extracted five times with equal volumes of ether. When the combined extracts were dried over magnesium sulfate and evaporated under reduced pressure, a white crystalline residue of 520 mg., 26.4% yield based on proposed formula for base, was obtained. The product melted at 158–160°; $[\alpha]^{25.8}D$ −20.5° (*c* 2.15%, in methanol). Three further extractions with ethyl acetate yielded 720 mg. additional material which proved to have the same melting point and rotation as the ether extractable material. The combined products were recrystallized from 20 ml. of hot water: (m. p. 161–162°); $[\alpha]^{26}D$ −23.1° (*c* = 1.58%, in methanol).

Anal. Calcd. for $C_9H_{12}O_4N_2$: C, 50.94; H, 5.69; N, 13.20. Found: C, 51.15; H, 5.80; N, 13.29.

The base was converted to the hydrochloride by dissolving in 20 ml. of 1.0 *N* hydrochloric acid and evaporating the solvent under reduced pressure at 40°. After one recrystallization from ethanol–low petroleum ether mixture, the product melted at 208–210°; $[\alpha]^{24.8}D$ −26.8°, (*c*, 3.27%, in methanol). The product was identical with that obtained by hot acid hydrolysis of chloramphenicol.

In another experiment an acid identified as dichloroacetic (see below) was obtained by extracting the acidified hydrolysate three times with acid-free ether before proceeding to the isolation of the base.

(10) All melting points were determined on a calibrated Fisher-Johns melting point block.

Identification of the Acid Fragment Obtained by Acid or Alkaline Degradation of Chloramphenicol. The Benzyl-S-thiuronium Salt.—The acid residues remaining from acid and alkaline degradations of chloramphenicol were converted to identical benzyl-S-thiuronium salts by the following procedure. The acidic residues isolated by evaporation of the ether extract of systems containing 5 g. of chloramphenicol which had been degraded with 1.0 *N* hydrochloric acid or 0.1 *N* sodium hydroxide as described above were dissolved in 1-ml. portions of distilled water and neutralized to *p*H 6.2 with 7% sodium bicarbonate. One milliliter of hot ethanol solution containing 150 mg. of S-benzyl-thiuronium chloride was added. The reaction mixture was warmed on the steam-bath for a minute, then allowed to cool to room temperature, and finally chilled in ice. The crystalline products isolated by filtration were recrystallized from absolute ethanol. The salts thus obtained melted at 180° and did not depress the melting point of an authentic dichloroacetic acid salt prepared in the same manner.

Anal. Calcd. for $C_{10}H_{12}O_2N_2Cl_2S$: C, 40.6; H, 4.1. Found: C, 40.73; H, 4.04.

The benzyl-S-thiuronium salt of dichloroacetic acid was previously described by Stig Veibel and Kaj Ottung,[11] m. p. 178–179°.

Acetylation of Chloramphenicol.—The antibiotic was acetylated by dissolving 300 mg. in a mixture of 2 ml. of dry pyridine and 2 ml. of acetic anhydride at 0°. The solution was kept cold for one hour and then allowed to warm to room temperature. After standing overnight the reaction mixture was poured into 70 ml. of ice-water and extracted three times with ether or ethyl acetate. The combined extracts were washed successively with 2% sulfuric acid, 2% sodium carbonate solution. and water, and dried over magnesium sulfate. The product obtained upon evaporation was recrystallized from 90% ethanol to a yield of 330 mg. of acetyl derivative (m. p. 141–142°).

Anal. Calcd. for $C_{15}H_{16}O_7N_2Cl_2$: C, 44.24; H, 3.96 N, 6.88; O-acetyl, 21.1. Found: C, 44.42; H, 4.15; N, 6.90; O-acetyl, 21.7.

The solution remaining from the Kunz determination for O-acetyl was extracted three times with ethyl acetate. The combined extracts were dried over magnesium sulfate and evaporated. Two recrystallizations of the residue from hot water yielded pure chloramphenicol (determined by mixed melting point and microbiological activity).

N-Acetyl Derivative of the Base Obtained by Acid or Alkaline Degradation of Chloramphenicol.—Acetylation of the base under a variety of conditions was investigated since isomerization in the ephedra and *nor*ephedra series has been demonstrated to occur under certain conditions. However, each procedure was found to yield the same product.

Procedure (a).—The following conditions have been found to cause no isomerization of ephedrine and *nor*-ephedrine isomers.[12,13] One gram of free base was treated with 3 ml. of acetic anhydride at 70° for fifteen minutes. The reaction mixture was cooled and 30 ml. of ice-water added. After neutralization with solid sodium bicarbonate. the ice-cold solution was extracted four times with ethyl acetate. The combined extracts were dried with magnesium sulfate and evaporated. Trituration of the gummy residue with 5 ml. of chloroform effected crystallization. The N-acetyl derivative (0.65 g.) recrystallized twice from 8 ml. of ethyl acetate melted at 125–126°. The product could also be isolated by evaporating the excess acetylation reagents *in vacuo* immediately following the 70° treatment and triturating the residue with chloroform.

Anal. Calcd. for $C_{11}H_{14}N_2O_5$: C, 51.97; H, 5.52; N, 11.03. Found: C, 51.98; H, 5.49; N, 11.15.

(11) Veibel and Ottung, *Bull. Soc. Chim.*, **6**, 1434 (1939).

(12) Mitchell, *J. Chem. Soc.*, 1153 (1940).

(13) Welsh, THIS JOURNAL, **69**, 128 (1947).

When the mother liquors from crystallization of the N-acetyl derivative were evaporated and the gummy residue treated according to the Kunz[4] procedure to remove O-acetyl, an additional quantity (0.41 g.) of the 125–126° acetate could be isolated by extracting the hydrolysate with ethyl acetate and evaporating the extract. It is thus shown that even under the mild conditions described some O-acetylation occurs.

Procedure (b).—One hundred milligrams of free base was treated with 1 ml. of acetic anhydride at 140–145° for fifteen minutes. The excess reagent was removed *in vacuo* and the gummy residue which failed to crystallize treated under Kunz conditions to remove O-acetyl. The neutralized reaction mixture was extracted three times with ethyl acetate and the extracts dried and evaporated. The product crystallized upon trituration with chloroform. After recrystallization from acetone–chloroform–low petroleum ether mixture. 70 mg. of product were obtained (m. p. 125–126°) which did not depress the melting point of the derivative obtained in procedure (a).

Procedure (c).—When 100 mg. of base hydrochloride was treated with 1 ml. of acetic anhydride at 140–150° for three hours, and the reaction mixture worked up as described in procedure (b) a yield of 60 mg. of product melting at 125–126° was obtained. This derivative was shown to be identical with procedure (a) and (b) products.

Triacetyl Derivative of Chloramphenicol Base.—One gram of the free base was treated with 5 ml. of acetic anhydride at 70° for fifteen minutes. The reaction mixture was then cooled to 0° and an equal volume of dry pyridine added. The reaction mixture was kept overnight at room temperature and then concentrated to a gum *in vacuo*. Trituration with ether induced crystallization. The product was recrystallized from ether–low petroleum ether mixture three times (m. p. 109–111°).

Anal. Calcd. for $C_{15}H_{18}O_7N_2$: C, 53.25: H, 5.36; N, 8.28. Found: C, 53.55; H, 5.43; N, 8.65.

The N-*p*-Nitrobenzoyl Derivative of the Base Obtained by Acid Degradation of Chloramphenicol.—The N-*p*-nitrobenzoyl derivative was prepared by shaking 560 mg. of the base hydrochloride with 430 mg. of *p*-nitrobenzoyl chloride in a mixture of 20 ml. of ice-cold 0.5 *N* potassium hydroxide and 40 ml. of ether for ten minutes. The crystalline amide separating from the reaction mixture was collected by filtration (yield, 710 mg.). After three recrystallizations from ethanol the product melted at 204–205°, $[\alpha]^{26}D$ −140.2° (*c* 1.96%, in dioxane).

Anal. Calcd. for $C_{16}H_{15}N_3O_7$: C, 53.18; H, 4.18; N. 11.63. Found: C. 53.36; H, 4.05: N, 11.78.

The Urethan of the Base Obtained by Acid Degradation of Chloramphenicol.—A quantity of 530 mg. of chloramphenicol base was suspended in a solution of 210 mg. of sodium bicarbonate in 10 ml. of distilled water and 0.30 ml. of ethyl chlorocarbonate added. The base went into solution and reaction product oiled out with evolution of carbon dioxide. After two hours at room temperature the reaction mixture was chilled in ice for one hour. The crystalline product was collected by filtration in a yield of 545 mg. (m. p. 104–106°). After three recrystallizations from ethylene dichloride the material was analyzed (m. p. 107–108°).

Anal. Calcd. for $C_{12}H_{16}N_2O_6$: C, 50.69; H, 5.67; N. 9.86. Found: C. 50.62; H. 5.69; N, 9.85.

The Phenyl Thiourea of the Base Obtained by Acid Degradation of Chloramphenicol.—To a suspension of 300 mg. of the free base in 4 ml. of absolute ethanol was added 0.2 ml. of phenyl isothiocyanate. The reaction mixture was allowed to stand at room temperature for forty-eight hours during which time the base dissolved. When the solution was made turbid by addition of low petroleum ether 420 mg. of crystalline product separated. The product was recrystallized three times from ethyl acetate–low petroleum ether (m. p. 160–161°).

Anal. Calcd. for $C_{16}H_{17}N_3O_4S$: C. 55.32; H. 4.93: N, 12.10. Found: C, 55.50; H, 4.80; N. 12.21.

Reconstitution of Chloramphenicol.—The free base (100 mg.) obtained from acid degraded chloramphenicol was heated on the steam-bath for one and one-half hours with 0.3 ml. of methyl dichloroacetate. The reaction mixture was cooled and diluted with low petroleum ether to remove excess ester. The solid residue melted at 134–139° with no depression of the melting point of the natural antibiotic. Two recrystallizations from ethyl acetate–low petroleum ether and a final crystallization from acetone–low petroleum ether yielded 70 mg. of pure chloramphenicol, (m. p. 150–151°), fully active *vs. Shigella paradysenteriae* (Sonnei).

The Reduction of the Aromatic Nitro Group of the Base Obtained by Acid or Alkaline Degradation of Chloramphenicol.—Three hundred milligrams of free base was hydrogenated for one hour in the presence of 100 mg. of reduced palladium oxide catalyst at atmospheric pressure. Water or 50% aqueous ethanol was employed as the solvent. A micro hydrogenation showed that under these conditions exactly three molecular equivalents of hydrogen were absorbed. At the end of the reaction the catalyst was filtered and aqueous residue concentrated at reduced pressure to a crystalline solid. The product was recrystallized twice from hot ethanol (m. p. 136–137°). The compound gave a positive diazotization-coupling reaction and showed characteristic absorption for a para-anilino-aromatic nucleus.

Anal. Calcd. for $C_9H_{14}N_2O_2$: C, 59.31; H, 7.74; N, 15.38. Found: C, 59.18; H, 7.43; N, 15.30.

Quantitative Periodate Studies.—A study of the periodate oxidation of systems of chloramphenicol, alkaline degraded chloramphenicol, and the isolated bases obtained by acid and alkaline degradation of the antibiotic was made. While chloramphenicol was shown to undergo no oxidation, the other systems consumed two molecular equivalents of oxidant. The reaction was virtually complete in ten minutes. The data are summarized in Table III.

TABLE III

PERIODATE OXIDATION DETERMINATIONS

System	mM. cpd. present	mM. HIO_4 present	mM. HIO_4 consumed (30 min.)	mM. HIO_4/mM. cpd.
(a) Chloramphenicol	0.0156	0.0633	0.001	0.06
(b) Chloramphenicol in 0.1 *N* NaOH. after 18 hours	.0157	.0482	.0277	1.77
(c) Base from 0.1 *N* NaOH treatment	.0189	.0460	.0361	1.91
(d) Base hydrochloride from 0.1 *N* HCl treatment	.0164	.0564	.0320	1.96

Procedure (a).—A sample of 40.3 mg. of chloramphenicol was dissolved in 40 ml. of standard periodic acid which had been neutralized to *p*H 7 with sodium bicarbonate. To a 5-ml. aliquot was added a measured volume of 0.00982 *N* arsenite, *ca.* 0.25 g. of sodium bicarbonate, and 0.5 ml. of 20% potassium iodide solution. After ten minutes the excess arsenite was titrated with 0.00952 *N* iodine. Aliquots were analyzed at intervals during a three-hour period. The above procedure was applied to systems (b), (c) and (d) as well. System (b) was neutralized to *p*H 7 with 0.5 *N* sulfuric acid before adding the periodate solution.

Products of the Periodate Oxidation of Base Obtained by Acid or Alkaline Degradation of Chloramphenicol.—A sample of 500 mg. of the base hydrochloride was treated with 1.5 g. of sodium periodate in 25 ml. of distilled water. After one hour, the solution was extracted three times with acid-free, aldehyde-free ether. (a) ***p*-Nitrobenzaldehyde.**—When the dried ether extract was evaporated, a crystalline residue of 304 mg. (m. p. 105–106°) was obtained. This product did not depress the melting point of *p*-nitrobenzaldehyde, 106°.

Anal. Calcd. for $C_7H_5O_3N$: C, 55.64; H, 3.34; N, 9.27. Found: C, 55.73; H, 3.59; N, 9.48.

The oxime and phenylhydrazone derivatives of the aldehyde had the same melting points as the corresponding derivatives of *p*-nitrobenzaldehyde and did not depress the melting points of these compounds.

Anal. Calcd. for $C_7H_6O_3N_2$: C, 50.60; H, 3.65; N, 16.86. Found: C, 50.50; H, 3.64; N, 17.18 (oxime, m. p. 129–130°). Calcd. for $C_{13}H_{11}O_2N_3$: C, 64.72; H, 4.60; N, 17.43. Found: C, 64.97; H, 4.78; N, 17.42 (phenylhydrazone, m. p. 159–160°).

(b) **Formaldehyde.**—The aqueous residue was next treated with 5 ml. of 1 *M* arsenite and 1 ml. of 20% potassium iodide solution. After fifteen minutes the *p*H was adjusted to 5 with glacial acetic acid and 6 ml. of dimedon reagent (c = 100 mg./ml. in 95% ethanol) added. An immediate precipitate formed. The product was filtered after two hours to a yield of 390 mg. of derivative melting at 189–190° with no depression of the melting point of formaldehyde dimedon.

The filtrate from the dimedon precipitate was made strongly alkaline with 20% sodium hydroxide and the solution distilled into 75 ml. of 0.1000 *N* sulfuric acid. The distillate was diluted to 250-ml. and a 100-ml. aliquot back-titrated with 0.1000 *N* sodium hydroxide. It was found that 15.00 ml. of 0.1000 *N* acid had reacted with the total volatile base. The usual Nesslerization procedure was applied to a 10-ml. aliquot and a total yield of 27.5 mg. of ammonia was found in the reaction.

Assuming that the reaction proceeds to molecular equivalents of *p*-nitrobenzaldehyde, formaldehyde, ammonia and formic acid, Table IV summarizes the yields obtained in practice.

TABLE IV

YIELDS OF PRODUCTS ISOLATED FROM PERIODATE DEGRADED BASE

Product	Mg. obtained	Theoretical yield, mg.	Yield, %
p-Nitrobenzaldehyde	304	304	100
Formaldehyde dimedon	390	452	86
Ammonia	30.5 (by titration)	34.2	89
	27.5 (by Nesslerization)		80
Formic acid	Not demonstrated		

Summary

The chemical structure of chloramphenicol (Chloromycetin) has been determined to be D-(−)-*threo* - 2 - dichloroacetamido - 1 - *p* - nitrophenyl-1,3-propanediol.

DETROIT, MICHIGAN RECEIVED DECEMBER 20, 1948