alanine ester was saponified by the addition of methanol (75 cc.) and 2 *N* potassium hydroxide solution (12.2 cc.). The methanol was evaporated on the steam-bath and the *p*H of the aqueous residue adjusted to about 6.5 by the addition of dilute acid. A precipitate of nicotinylphenylalanine was obtained which crystallized from water in the form of short, colorless needles, 2.9 g. (86%), m.p. 198°.

Anal. Calcd. for $C_{15}H_{14}O_3N_2$: C, 66.71; H, 5.22. Found: C, 66.63; H, 5.23.

Nicotinylphenylalanine was also prepared by adding powdered nicotinic acid chloride hydrochloride (3.2 g.) to a solution of phenylalanine (2.7 g.) in 2 *N* sodium hydroxide solution (8.23 cc.) and water (100 cc.), slowly and with stirring, simultaneously with a solution of sodium carbonate (4 g.) in water (50 cc.). The mixture was stirred for a further 30 minutes, the *p*H adjusted to about 7 and the solution concentrated to about 20 cc. under reduced pressure. The solid which crystallized on cooling was collected and digested with boiling alcohol and filtered. The residue consisted of phenylalanine. Nicotinylphenylalanine 1.8 g. (43%) crystallized from the filtrate upon concentration and cooling.

Nicotinyl-DL-phenylalanylhydrazide.—Nicotinylphenylalanine ethyl ester (1 g.) was heated on the steam-bath for 1.5 hours with 85% hydrazine hydrate (10 cc.). Ether (50 cc.) was then added to the cooled reaction mixture, whereupon the hydrazide precipitated. It was collected by filtration and recrystallized from a mixture of alcohol and benzene, 0.7 g. (72%), m.p. 185°.

Anal. Calcd. for $C_{15}H_{16}O_2N_4$: C, 63.36; H, 5.68. Found: C, 63.42; H, 6.28.

N-(Benzylnicotinamidomethyl)-N'-β-naphthylurea (XIV).—To an ice-cold solution of nicotinylphenylalanylhydrazide (0.5 g.) in 2 *N* hydrochloric acid (20 cc.) was added slowly a cooled solution of sodium nitrite (0.15 g.) in water (5 cc.). The resulting solution was kept at room temperature for 15 minutes, cooled in ice and neutralized by the addition of solid sodium bicarbonate. The precipitated nicotinylphenylalanyl azide (XII) was extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulfate. This solution, after addition to it of β-naphthylamine (0.25 g.), was kept at room temperature for 48 hours. The solvent was then removed under reduced pressure and the solid residue digested with boiling water and filtered hot. The filtrate contained unreacted naphthylamine. The residue was recrystallized from alcohol. The urea derivative (XIV) crystallized in colorless flakes, 0.35 g. (48%), m.p. 227°.

Anal. Calcd. for $C_{25}H_{22}O_2N_4$: C, 73.14; H, 5.41. Found: C, 73.12; H, 4.99.

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[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC.]

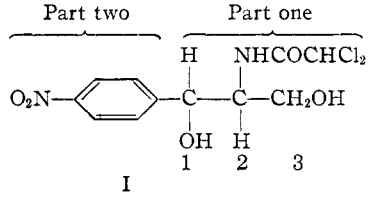
The Synthesis of Chloramphenicol Analogs

BY CHARLES F. HUEBNER AND CAESAR R. SCHOLZ

The acid related to chloramphenicol by oxidation at the primary alcoholic grouping has been synthesized. As a by-product in the saponification of N-dichloroacetyl-O-acetyl-*p*-nitrophenylserine ethyl ester, the alkaline soluble α-dichloroacetamido-*p*-nitrocinnamic acid ethyl ester was obtained. A homochloroamphenicol, 1-(*p*-nitrophenyl)-2-dichloroacetamido-2-methyl-1,3-propanediol, was prepared. By reduction of 1,3-diphenyl-2-phenylhydrazono-1,3-propanedione and subsequent transformations, 1,3-di-(*p*-nitrophenyl)-2-dichloroacetamido-1,3-propanediol was synthesized. Four compounds containing a *p*-nitrophenyl grouping and a polyhydroxy side chain were also prepared. None of these synthetic analogs of chloramphenicol showed any antibiotic activity.

The characterization of chloramphenicol (I) and the elaboration of two methods for its synthesis by Parke, Davis and Co. chemists[1,2,3,4] makes this antibiotic the first one in which the relation between structure and biological action can be thoroughly explored. For purposes of discussion one may divide the chloramphenicol molecule (I) into two parts; first, the stereochemically specific 2-acylamido propanediol side chain, which can be regarded as a grouping native to physiological systems in the same sense that penicillin is peptide-like and streptomycin is carbohydrate-like and, second, the nitrobenzene grouping which is more in the nature of the classical chemotherapeutic agent. Controulis, *et al.*,[2] have already shown that only one of the four possible side chain stereoisomers of chloramphenicol is active. Changes in the side chain considered in this paper result in complete loss of antibiotic activity.

On the other hand it is becoming evident that such high structural specificity in the aromatic grouping is not necessary for antibiotic activity. Long, *et al.*, Bambas, *et al.*,[5] and Buu-Hoï[6] have reported that the *m*- and *o*-nitro isomers of I and the analogs with a halogen replacing the nitro group exhibit appreciable activity. Forthcoming work from this Laboratory will further substantiate this generalization.



I

The first change in the side chain of I to be discussed is the replacement of the primary alcohol group at C_3 by a carboxyl group. The starting material used was the DL-phenylserine (II) of Erlenmeyer[7] formed as the major diastereomer in the reaction between glycine and benzaldehyde in alkaline solution. Although the configuration of this amino acid was allegedly shown to be "*trans*" by Forster and Rao,[8] thus corresponding to the *threo*-configuration of I, the proof is not conclusive. To demonstrate that I and II are of the same configuration, we have reduced the methyl ester of II with lithium aluminum hydride to an amino alcohol identical with DL-*threo*-1-phenyl-2-amino-1,3-propanediol which can be converted to I as described by Long and Troutman.[3] The com-

(1) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, THIS JOURNAL, **71**, 2458 (1949).

(2) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., *ibid.*, **71**, 2463 (1949).

(3) L. M. Long and H. D. Troutman, *ibid.*, **71**, 2469 (1949).

(4) L. M. Long and H. D. Troutman, *ibid.*, **71**, 2473 (1949).

(5) L. M. Long and N. Jeneseł, *ibid.*, **72**, 4299 (1950); L. L. Bambas, H. D. Troutman and L. M. Long, *ibid.*, **72**, 4445 (1950).

(6) Buu-Hoï, Hoán, P. Jacquignon and N. H. Khoi, *Compt. rend.*, **230**, 662 (1950).

(7) E. Erlenmeyer, Jr., and E. Früstück, *Ann.*, **284**, 36 (1895).

(8) M. O. Forster and K. A. N. Rao, *J. Chem. Soc.*, 1943 (1926).

parison was made through the N-benzoates (III). Since the completion of these experiments, we have noted similar conclusions regarding the configuration of Erlenmeyer's phenylserine by Carrara and Weitnauer[9] and by Shaw and Fox.[10]

The ethyl ester of II was N-dichloroacetylated (IV), O-acetylated (V) and nitrated (VI). VI, when treated with two molar equivalents of base in the cold to minimize any possible epimerization at C_2, yielded the N-dichloroacetyl-*p*-nitrophenylserine (VII). An interesting side product formed during the hydrolysis of VI to VII was α-dichloroacetamido-*p*-nitrocinnamic acid ethyl ester (VIII). The loss of acetic acid and the formation of the water-soluble sodium salt of VIII account for the consumption of the two molar equivalents of sodium hydroxide. The somewhat surprising acidic nature of VIII can be explained by the combined presence in the molecule of the nitro catio-enoid system and the positive inductive effect of the chlorine atoms. The compound obtained by replacing the dichloroacetyl group by an acetyl group, α-acetamido-*p*-nitrocinnamic acid methyl ester (IX), partially dissolves in one molar equivalent of sodium hydroxide, indicating that the nitro group alone confers some acidic properties to the compound. It is not as strongly acidic as VIII, however, since the latter readily dissolves completely in alkali under the same conditions. IX also suffers some saponification to the acid X but on acidifying the solution, a certain amount of IX is recovered. X was prepared by nitration of 2-methyl-4-benzaloxazolone (XI). The former by treatment with diazomethane yielded IX.

The structure of VIII was indicated by the presence of an ethoxyl, by its saponification to the corresponding acid XII, and by its acid hydrolysis to *p*-nitrophenylpyruvic acid (XIII) with loss of the amino group. By contrast, VII, when subjected to similar acid hydrolysis, gave an intractable mixture from which only a small amount of crystalline material melting at 160–170° could be obtained. Suffice it to say that the course of hydrolysis of VII and VIII is different.

The similarity of the ultraviolet absorption spectrum of VIII to that of the authentic cinnamic acid derivative (IX) (Fig. 1) as compared to the phenylserine derivative lacking the additional double bond in the side chain (VI) is corroborative evidence for the structure of VIII.

Even though there are five functional groups in VI which can react with lithium aluminum hydride, it was considered of interest to attempt to reduce VI to DL-chloramphenicol with limited amounts of this reagent. A chromatographic fraction of the sirupy reaction mixture, although non-crystalline, possessed antibiotic activity against *S. paradysenteriae* equal to DL-chloramphenicol. The major part of the said fraction is presumably the latter substance. It has been shown by Felkin[11] that the ester group of ethyl *p*-nitrobenzoate and related compounds can be preferentially reduced to the primary alcohol by lithium aluminum hydride without affecting the nitro group.

While preparing this manuscript, the synthesis of VII with a reported melting point of 158–162° from phenylserine by a different sequence of reactions was reported by Woolley.[12] Our preparation of VII, however, melted considerably higher (173–175°). The question of identity of these two substances is discussed in the experimental section.

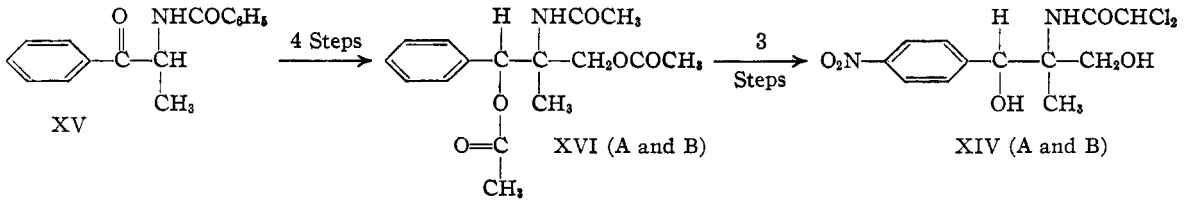
A second biologically inactive analog of I was synthesized in which a methyl group is substituted for the hydrogen of C_2. The sequence of reactions used to prepare this homochloramphenicol (XIV) was similar to the excellent and well described method of the Parke, Davis group.[3] α-Benzamidopropiophenone (XV) was condensed with formaldehyde, reduced, hydrolyzed and acetylated. A separation into the two possible racemates of the triacetate (XVI) (designated as A and B since the configurations are not known)

(9) G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949).

(10) K. W. F. Shaw and S. W. Fox, Abstracts of Papers, American Chemical Society, Sept. 3, 1950, p. 28N.

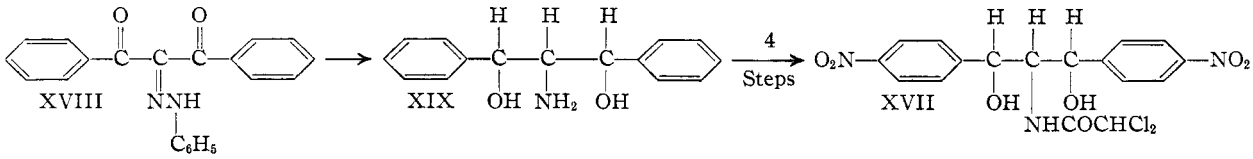
(11) H. Felkin, *Compt. rend.*, **230**, 304 (1950).

(12) D. W. Woolley, *J. Biol. Chem.*, **185**, 293 (1950).



was made possible by the fact that modification A crystallized and was virtually insoluble in ether while the modification B was an ether-soluble sirup. Nitration, hydrolysis and N-dichloroacetylation of each modification completed the synthesis to yield the two crystalline DL-mixtures of XIV.

The third analog of I described in this paper is a bis-type molecule in which a *p*-nitrophenyl group replaces an H on C_3 (XVIII). The synthesis began with 1,3-diphenyl-2-phenylhydrazono-1,3-propanedione (XVIII). After a number of unsuccessful attempts catalytically to reduce XVIII to the amino alcohol XIX in acid media, Raney nickel in ethanol–ethyl acetate was found to work satisfactorily. Apparently, the ketone groups



are reduced before the amino group is generated or a dihydropyridazine would be the expected product. N-Dichloroacetylation, O-acetylation, nitration and selective hydrolysis of the O-acetyl groups lead to the final product, XVII. The stereochemical configuration of this molecule is unknown. Only one amino alcohol (XIX) could be isolated from the reduction mixture.

The *p*-position of the nitro group in all of these analogs, VII, XVA and B, and XVII was determined by oxidation to *p*-nitrobenzoic acid.

A fourth miscellaneous group of substances, XX, XXI and XXIII, more distantly related to I in which the side chain is carbohydrate-like in nature and bears an amino nitrogen was synthesized. N-Phenyl-D-mannamine was nitrated after protection of the hydroxyls by acetylation. Deacetylation yielded the crystalline *p*-nitro-derivative XX. N-Dichloroacetylation of this substance failed apparently because of the weakly basic nature of the secondary amino group. The nitro group is assigned the *p*-position because of the similarity in absorption spectra of XX and *p*-nitroaniline as contrasted to *o*-nitroaniline (Fig. 2).

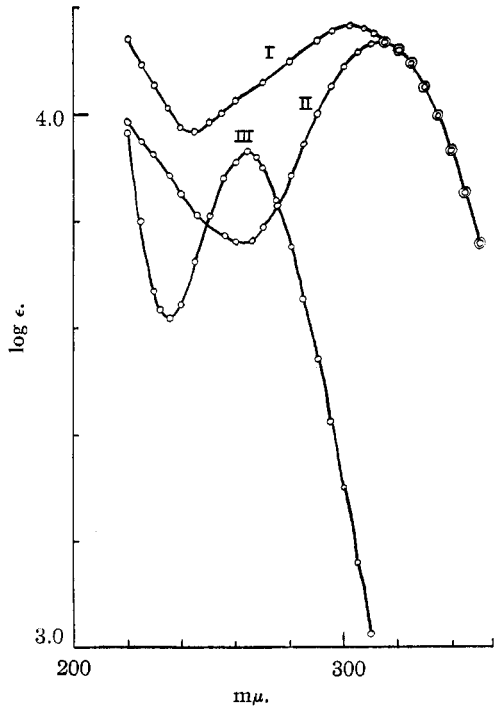


Fig. 1.—I, α-Dichloroacetamido-*p*-nitrocinnamic acid ethyl ester; II, α-acetamido-*p*-nitrocinnamic acid methyl ester; III, N-dichloroacetyl-O-acetyl *p*-nitrophenylserine ethyl ester. Solvent: ethanol.

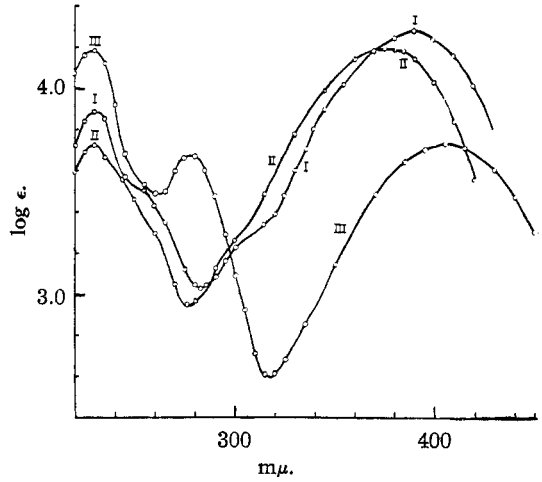
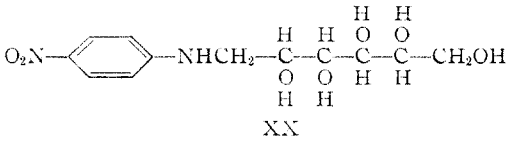


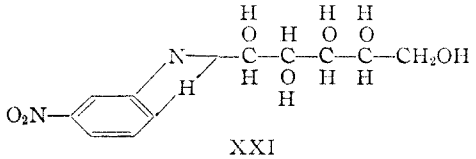
Fig. 2.—I, N-(D-*manno*-pentahydroxyamyl)-*p*-nitroaniline; II, *p*-nitroaniline; III, *o*-nitroaniline. Solvent: ethanol.

2-(D-*gluco*-Pentahydroxyamyl)-5-nitrobenzimidazole (XXI) was prepared by acid-catalyzed condensation of D-gluconic acid with 2-amino-4-nitroaniline.[13] The synthesis of N-glycosides of glucosamine has never been described. Through the use of N-acetyl-D-glucosamine, condensation with aniline and with *p*-nitroaniline was accomplished under conditions similar to those used to prepare the corresponding glucosides.[14]

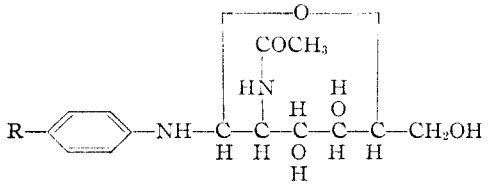
(13) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 293 (1940).
(14) F. Weygand, *Ber.*, **72**, 1663 (1939).



XX

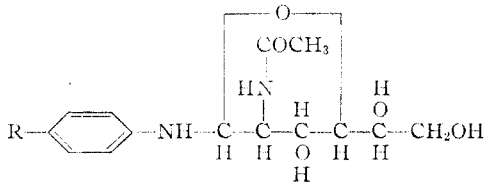


XXI



XXII

(a) R = H, (b) R = NO_2



XXIII

(a) R = H, (b) R = NO_2

An attempt was made to establish the ring structure (XXIIa or XXIIIa) of the aniline condensation product. Since on acetylation a tri- rather than a tetracetate was obtained, the Schiff base type structure is ruled out. In attempting to apply the periodate oxidation technique to this problem, it was found that within one minute the anilide consumed over three molar equivalents of sodium metaperiodate with precipitation of a brown amorphous material indicating complete disruption of the ring system. This type of reaction has been found with many arylamine-N-glycosides.[15] When XXIIa (or XXIIIa) was carefully treated with one molar equivalent of sodium periodate, a 38% yield of formaldehyde (as the dimedon condensation product) was obtained even though no attempt was made to achieve a quantitative isolation. This observation leads us to prefer the furanoside structure XXIIIa (and XXIIIb by analogy). The difficulties of assigning the ring structure to compounds of this type are discussed by Honeyman and Tatchell.[16]

No antibiotic activity was shown by VII, XIV, XVII, XX, XXI or XXIIIb. The assays were kindly carried out under the supervision of Dr. R. L. Mayer of the Microbiological Division of Ciba Pharmaceutical Products, Inc. The authors wish to acknowledge the capable assistance of Mr. P. A. Diassi and Mrs. K. Oney and express thanks to Mr. L. Dorfman for the microanalytical data. Dr. K. Hofmann first suggested the structure of VIII.

(15) L. Berger and J. Lee, *J. Org. Chem.*, **11**, 75 (1946).

(16) J. Honeyman and A. R. Tatchell, *J. Chem. Soc.*, 967 (1950).

Experimental[17]

DL-*threo*-**1-Phenyl-2-benzamido-1,3-propanediol (III) from DL-Phenylserine (II).**—An ethereal solution of diazomethane was added in portions with occasional stirring to 30 g. of phenylserine (II)[8] (m.p. 200–202°) suspended in 100 ml. of ethanol–water (3–1) until all the II had dissolved and a permanent yellow color remained. The sirup resulting after removal of the solvent *in vacuo* was dissolved in 70 ml. of dioxane and 100 ml. of dry ether added. This solution was dropped with stirring into a flask containing 30 g. of lithium aluminum hydride in 250 ml. of ether. After refluxing for 3 hours, water was added dropwise to decompose excess reagent to the easily filtered lithium aluminate. The latter was extracted repeatedly with hot water and separated from the gelatinous alumina by centrifugation. The 10 g. of sirup remaining on evaporation of the aqueous extract was redissolved in water and benzoylated by the Schotten–Baumann technique. By shaking at room temperature for 12 hours with excess alkali, any O-benzoate was saponified. The semicrystalline material resulting was recrystallized from ethanol–water (1–1); yield 6.6 g. of fine needles of III, m.p. 166–167°.

No exhaustive attempts were made to increase the yield of III. A sample of III synthesized according to Long and Troutman[3] melted at 165–166° and a mixture of the two substances melted at 166–167°. The identity was confirmed by the infrared absorption spectra kindly carried out by Dr. K. Dobriner of the Sloan–Kettering Institute for Cancer Research.

Anal. Calcd. for $C_{16}H_{17}O_3N$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.55; H, 6.18; N, 5.12.

N-Dichloroacetyl Phenylserine Ethyl Ester (IV).—II was converted to its ethyl ester hydrochloride by the usual method. To a suspension of 10 g. of the ester hydrochloride in 100 ml. of cold ethyl acetate and 100 ml. of ice-water in a separatory funnel was added 7 ml. of 5.9 *N* sodium hydroxide. Dichloroacetyl chloride (3.8 ml.) was added quickly to the well shaken mixture followed by 8 g. of sodium bicarbonate added in portions. A further addition of 3.8 ml. of the acyl chloride and bicarbonate was made until the aqueous phase was basic. The crystalline product separating from the organic layer was collected (7.2 g.). By concentration of the ethyl acetate an additional 4 g. was obtained. Recrystallization from the ethanol gave 10.5 g. of IV, m.p. 149–150°.

Anal. Calcd. for $C_{13}H_{15}Cl_2NO_4$: C, 48.74; H, 4.69; Cl, 22.19. Found: C, 48.47; H, 4.91; Cl, 21.64.

N-Dichloroacetyl-O-Acetyl Phenylserine Ethyl Ester (V).—One gram of IV dissolved in 5 ml. of pyridine was treated with 5 ml. of acetic anhydride. After standing overnight the solution was concentrated to dryness *in vacuo* and the residue recrystallized from ethanol; m.p. 183–185°, yield 0.9 g.

Anal. Calcd. for $C_{15}H_{17}Cl_2NO_5$: C, 49.71; H, 4.73. Found: C, 49.67; H, 4.88.

N-Dichloroacetyl-O-acetyl *p*-Nitrophenylserine Ethyl Ester (VI).—One gram of V was slowly added to a stirred solution of 2 ml. of mixed acid (1 part by volume of sulfuric acid to 1 part of fuming nitric acid) held at 0°. The solution was allowed to warm up to room temperature and after one-half hour poured into ice-water. The oil crystallized on scratching. Recrystallization from ethanol–water gave 0.9 g. of VI, m.p. 108–109°.

Anal. Calcd. for $C_{15}H_{16}Cl_2N_2O_7 \cdot {}^1/_2H_2O$: C, 43.27; H, 4.09. Found: C, 43.25; H, 3.94.

***p*-Nitrobenzoic Acid from VI.**—VI (1.0 g.) dissolved in 30 ml. of pyridine–water (2–1) was heated on the steam-bath and 4.0 g. of powdered potassium permanganate added over the course of one-half hour. After an additional one-half hour heating, the excess permanganate was destroyed with sodium bisulfite and the filtrate concentrated to dryness. The *p*-nitrobenzoic acid separating after acidification of an aqueous solution of the residue was recrystallized from water, m.p. 239–240°, yield 0.22 g. No depression in melting point of the mixture with an authentic sample was noted.

Anal. Calcd. for $C_7H_5NO_4$: N, 8.38. Found: N, 8.43.

(17) All melting points are uncorrected and were taken in capillary tubes.

α-Dichloroacetamido-*p*-nitrocinnamic Acid Ethyl Ester (VIII) and N-Dichloroacetyl *p*-Nitrophenylserine (VII).—To 2 g. of VI in 10 ml. of methanol at 5° was added 1.75 ml. of 5.9 *N* sodium hydroxide. After standing in the refrigerator overnight, the methanol was removed *in vacuo* and the residue dissolved in 25 ml. of water. On acidification with hydrochloric acid 0.4 g. of fine needles of VIII was collected. After recrystallization from ethanol–water or water (in which the substance is sparingly soluble) the m.p. was 151–155°. When VIII was shaken with one molar equivalent of 0.1 *N* sodium hydroxide, immediate solution occurred and on acidification VIII was recovered.

Anal. Calcd. for $C_{13}H_{12}Cl_2N_2O_5$: C, 44.95; H, 3.46; Cl, 20.52; N, 8.07; OC_2H_5, 12.96. Found: C, 44.72; H, 3.57; Cl, 20.52; N, 8.36; OC_2H_5, 12.96.

The aqueous filtrate from VIII was evaporated to a few ml. *in vacuo* and 1.1 g. of crystalline material was collected. It could be recrystallized from a small volume of water or from ethyl acetate–petroleum ether, m.p. 173–175°.

Anal. Calcd. for $C_{11}H_{10}Cl_2N_2O_6$: C, 39.16; H, 2.97; Cl, 21.07. Found: C, 39.15; H, 3.30; Cl, 21.05.

Dr. D. W. Woolley kindly placed at our disposal a small sample of "N-dichloroacetyl-β-hydroxy-*p*-nitrophenylalanine," m.p. 158–162° (hot stage)[12] presumably identical with VII; we found the capillary melting point to be at 155–160°. The mixture of this preparation with VII (m.p. 173–175°) melted at 158–165°. VII crystallizes from water in hexagonal platelets, whereas Woolley's substance crystallized in tiny needles. These differences might be due to the fact that the phenylserine used as the starting material by Woolley was not free from *allo*-phenylserine[10] or that his final product contains small amounts of XII.

Reduction of N-Dichloroacetyl-O-acetyl-*p*-nitrophenylserine Ethyl Ester (VI).—To a stirred solution of 3 g. of VI in 350 ml. of dry ether was added 5 ml. of a 2 *M* solution of lithium aluminum hydride in ether. After agitating for one-half hour, the insoluble metal complex was decomposed with 30 ml. of 2 *N* sulfuric acid. The ether phase yielded 1.45 g. of a sirup. This was redissolved in ether and chromatographed on 20 g. of alumina (Alorco). Material eluted with 400 ml. of ether showed no antibiotic activity. The eluate of 250 ml. of ethanol yielded 0.5 g. of a gummy substance which could not be induced to crystallize. When tested against *S. paradysenteriae*, it showed an activity approximately one-half of that of chloramphenicol.

α-Dichloroacetamido-*p*-nitrocinnamic Acid (XII).—VIII (0.029 g.) in 18 ml. of 0.1 *N* sodium hydroxide was held at 40° for two hours. The needles forming on acidification with hydrochloric acid were filtered and recrystallized from water; yield 0.015 g., m.p. 240–242°.

Anal. Calcd. for $C_{11}H_8Cl_2N_2O_5$: C, 41.35; H, 2.53. Found: C, 41.59; H, 3.02.

***p*-Nitrophenylpyruvic Acid (XIII) from VIII.**—VIII (0.2 g.) was refluxed for two hours with 2 *N* hydrochloric acid in 50 ml. of ethanol–water (1–1). The residue obtained by concentration to dryness *in vacuo* was recrystallized from water; yield 0.04 g., m.p. 195–200° (dec.). Reissert reported the melting point as 194° with previous sintering.[18]

Anal. Calcd. for $C_9H_7NO_5$: C, 51.67; H, 3.35; N, 6.74. Found: C, 51.54; H, 3.19; N, 6.82.

α-Acetamido-*p*-nitrocinnamic Acid (X) and Methyl Ester (IX).—One gram of 2-methyl-4-benzaloxazolone[19] (XI) was slowly added to 5 ml. of mixed acid at 0°. After warming up to room temperature, the mixture was poured into ice-water and the resulting precipitate (X) was recrystallized from water; yield 0.66 g., m.p. 243–245°.

Anal. Calcd. for $C_{11}H_{10}N_2O_5$: N, 11.20. Found: N, 11.20.

One gram of this acid methylated by diazomethane yielded 0.90 g. of fine needles of IX, m.p. 175–176°.

Anal. Calcd. for $C_{12}H_{12}N_2O_5$: N, 10.61. Found: N, 10.62.

IX (0.1 g.) was shaken with one molar equivalent of 0.1 *N* sodium hydroxide for one minute and filtered from 0.07 g. of unchanged IX. The filtrate was immediately acidified and the mixture of the acid (X) and its methyl ester IX was filtered, m.p. 180–195°. Analysis shows this mixture to consist of about one-third IX.

Anal. Calcd. for $C_{11}H_{10}N_2O_5$: OCH_3, 0.0; $C_{12}H_{12}N_2O_5$: OCH_3, 11.74. Found: OCH_3, 4.43.

α-Benzamido-β-hydroxyisobutyrophenone.—To 10 g. of α-benzamidopropiophenone (XV)[20] dissolved in 30 ml. of ethanol was added 3.1 g. of paraformaldehyde and 0.85 g. of potassium carbonate. While shaking at room temperature for several hours, the paraformaldehyde went into solution. After a reaction time of four hours, the mixture was poured into water and acidified with hydrochloric acid. The resulting gummy material soon solidified. Following crystallization from methanol–water, the melting point was 115–120°, wt. 9.2 g.

Anal. Calcd. for $C_{17}H_{17}NO_3$: C, 72.05; H, 6.04. Found: C, 72.28; H, 6.01.

The O-acetate was obtained by treatment with acetic anhydride–pyridine, m.p. 114–115°.

Anal. Calcd. for $C_{19}H_{19}NO_4$: C, 70.11; H, 5.85. Found: C, 70.66; H, 5.76.

1-Phenyl-2-amino-2-methyl-1,3-propanediol Triacetate (XVIA and B).—Twenty grams of α-benzamido-β-hydroxyisobutyrophenone was hydrogenated over Raney nickel at 45 p.s.i. and room temperature. In four hours, one molar equivalent of hydrogen had been adsorbed. The methanol and catalyst were removed and the resulting sirup hydrolized for four hours with 300 ml. of 6 *N* hydrochloric acid. The benzoic acid was extracted with ether and the aqueous phase concentrated to dryness *in vacuo*. The sirup was made alkaline with 5 *N* potassium hydroxide, saturated with potassium carbonate and extracted repeatedly with ethyl acetate. The extract was dried over sodium sulfate and concentrated to a sirup which was dissolved in 50 ml. of pyridine and 30 ml. of acetic anhydride. After standing overnight at room temperature, the solvents were removed *in vacuo*, the sirup taken up in chloroform and washed in turn with sodium bicarbonate, dilute hydrochloric acid and water. The sirup obtained after removal of the chloroform was dissolved in 200 ml. of ether. Crystallization of XVIA soon occurred. It was filtered and washed free of the sirupy XVIB with ether. Recrystallization of XVIA from ethanol gave 4.5 g. of plates, m.p. 163–165°.

Anal. Calcd. for $C_{16}H_{21}NO_5$: C, 62.50; H, 6.14. Found: C, 62.56; H, 6.74.

Evaporation of the ether mother liquor and washings yielded 8.5 g. of a sirup containing largely XVIB.

1-(*p*-Nitrophenyl)-2-amino-2-methyl-1,3-propanediol (A and B).—Fifteen grams of XVIA was nitrated as previously described for compound VI. The reaction mixture was poured into ice and the sirup extracted with ethyl acetate. The residue remaining after removal of the solvent was hydrolyzed for two hours with 200 ml. of 1.5 *N* hydrochloric acid on a steam-bath. The hydrolysis was conducted in a closed flask and with frequent shaking. The clear solution was concentrated to a small volume *in vacuo* when crystallization of the hydrochloride of 1-(*p*-nitrophenyl)-2-amino-2-methyl-1,3-propanediol (A) occurred; yield 6.2 g., m.p. 265° (after recrystallization from ethanol–water). Further amounts could be obtained from the mother liquor.

Anal. Calcd. for $C_{10}H_{14}N_2O_4 \cdot HCl$: C, 45.91; H, 5.71. Found: C, 46.01; H, 6.60.

The free base was obtained by suspending the hydrochloride in a little water and adding concentrated ammonia. It was recrystallized from water, m.p. 167–169°.

Anal. Calcd. for $C_{10}H_{14}N_2O_4$: C, 53.07; H, 6.20. Found: C, 52.67; H, 6.46.

Nitration of the sirup, XVIB, subsequent working up of the reaction mixture, and hydrolysis were carried out as described above to give in comparable yield, the hydrochloride of 1-(*p*-nitrophenyl)-2-amino-2-methyl-1,3-propanediol (B), m.p. 215–216°. The **free base** melted at 148–149° and the melting point of a mixture with the free base (A) was 135–140°.

Anal. Calcd. for $C_{10}H_{14}N_2O_4$: C, 53.07; H, 6.20. Found: C, 52.91; H, 5.87.

Oxidation of both of these bases carried out as described above yielded *p*-nitrobenzoic acid, m.p. 238–239°.

1-(*p*-Nitrophenyl)-2-dichloroacetamido-2-methyl-1,3-propanediol (XIVA and B).—One gram of 1-(*p*-nitrophenyl)-2-amino-2-methyl-1,3-propanediol (A) was heated on the

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(20) J. Lister and R. Robinson, *J. Chem. Soc.*, **101**, 1297 (1912).

steam-bath with 6 ml. of methyl dichloroacetate for one hour. The excess dichloroacetate was removed by washing with petroleum ether. The residue crystallized after several days in the refrigerator, yield 1.1 g. Recrystallization from either ethyl acetate or from water gave a pure XIVA, m.p. 153–154°.

Anal. Calcd. for $C_{12}H_{14}Cl_2N_2O_5$: C, 42.63; H, 4.15. Found: C, 42.57; H, 3.85.

Similarly, 1-(*p*-nitrophenyl)-2-amino-2-methyl-1,3-propanediol (B) was treated with methyl dichloroacetate. The sirupy product crystallized from water in plates. The substance (XIVB) partly melts at 110–120°, resolidifies and melts at 127–128°.

Anal. Calcd. for $C_{12}H_{14}Cl_2N_2O_5$: C, 42.63; H, 4.15. Found: C, 42.88; H, 4.37.

1,3-Diphenyl-2-amino-1,3-propanediol (XIX).—Two grams of 1,3-diphenyl-2-phenylhydrazono-1,3-propanedione (XVIII)[21] dissolved in a mixture of 100 ml. of ethanol and 20 ml. of ethyl acetate was hydrogenated over 0.3 g. of Raney nickel catalyst at 40 p.s.i. Filtration of the catalyst and evaporation of the solvent gave a paste of crystalline XIX and aniline. The latter was removed by repeatedly washing with petroleum ether. After recrystallization from ethanol–water, 1.1 g. of needles was obtained, m.p. 128–130°.

Anal. Calcd. for $C_{15}H_{17}NO_2$: C, 74.00; H, 7.08. Found: C, 73.88; H, 6.95.

1,3-Diphenyl-2-dichloroacetamido-1,3-propanediol.—XIX (0.3 g.) was heated with 1 ml. of methyl dichloroacetate for two hours on the steam-bath. On cooling crystallization occurred. Recrystallization from ethanol–water yielded 0.28 g., m.p. 172–175°.

Anal. Calcd. for $C_{17}H_{17}Cl_2NO_3$: N, 3.95; Cl, 20.06. Found: N, 3.94; Cl, 20.48.

Treatment with acetic anhydride and pyridine yielded the **diacetate**, m.p. 148–149°.

Anal. Calcd. for $C_{21}H_{21}Cl_2NO_5$: N, 3.19; Cl, 16.20. Found: N, 3.36; Cl, 16.13.

1,3-Di-(*p*-nitrophenyl)-2-dichloroacetamido-1,3-propanediol (XVII).—Two grams of the diacetate described above was added with stirring to 15 ml. of mixed acid held at 0°. Solution did not occur until the suspension had warmed up to room temperature. After an additional one-half hour, the mixture was poured onto ice, the amorphous precipitate collected, washed well with water and dried. One gram of the resulting powdery material was dissolved in 5 ml. of methanol and 3.8 ml. of 1 *N* sodium hydroxide added. The mixture was heated at 40° for two hours. During this time crystalline material appeared. After cooling it was collected and recrystallized from ethanol–water; m.p. 94–96°, yield 0.61 g.

Anal. Calcd. for $C_{17}H_{15}Cl_2N_3O_7$: C, 45.92; H, 3.42; N, 9.43; Cl, 15.98. Found: C, 45.70; H, 3.68; N, 9.52; Cl, 16.15.

Oxidation of 0.5 g. of XVII as described above gave 0.08 g. of *p*-nitrobenzoic acid, m.p. 230–239°, with no evidence of possible isomers.

N-(D-*manno*-Pentahydroxyamyl)-*p*-nitroaniline (XX).—To N-phenyl-D-mannamine[22] (3.0 g.) dissolved in 15 ml. of pyridine was added 15 ml. of acetic anhydride. After standing overnight at room temperature, the mixture was poured into ice-water and after two hours extracted three times with chloroform. The latter was washed successively with dilute hydrochloric acid, sodium bicarbonate and water, dried and evaporated to a sirup. Three ml. of fuming nitric acid (d. 1.5) was added to the solution of this sirup in 20 ml. of acetic acid and 1 ml. of acetic anhydride. After one hour at room temperature, the reaction mixture was poured onto ice water. This solution was extracted continually for two days with ether, the ether washed with sodium bicarbonate and dried over sodium sulfate and concentrated to dryness. The resulting sirup (3.91 g.) was dissolved in 25 ml. of chloroform and 19.0 ml. of 1.63 *N* sodium methoxide in methanol added at 0°. After standing overnight at 5°, the mixture was neutralized with hydrochloric acid and concentrated to dryness. The residue was recrystallized three times from water to yield 0.89 g. of yellow needles; m.p. 186–187°, $[\alpha]^{25}D$ +58.9° (*c*, 2 in pyridine).

Anal. Calcd. for $C_{12}H_{15}N_2O_7$: C, 47.65; H, 5.96; N, 9.26. Found: C, 47.78; H, 5.63; N, 8.64.

2-(D-*gluco*-Pentahydroxyamyl)-5-nitrobenzimidazole Hydrochloride (XXI).—A mixture of 20 g. of a 50% aqueous solution of D-gluconic acid, 8.6 g. of 1,2-diamino-4-nitrobenzene and 4.5 ml. of concentrated hydrochloric acid was heated in an open flask at 135° for two hours allowing the water to boil off. The resulting sirup was dissolved in 200 ml. of water, treated with norite and made basic with ammonia yielding a gel. This was dissolved by heating and on slowly cooling a gel was formed which gradually shrank from the walls to allow decantation of the mother liquors. This process was repeated twice and the residue dissolved in 50 ml. of 1 *N* hydrochloric acid. Crystals of XXI deposited on allowing the solution to evaporate at room temperature over a period of a week. On recrystallization from 95% ethanol–ether, 2.2 g. of fine, colorless needles of XXI were obtained; m.p. 206–207°, $[\alpha]^{24}D$ +6.0° (*c*, 2 in water).

Anal. Calcd. for $C_{12}H_{15}N_3O_7 \cdot HCl$: N, 12.01. Found: N, 11.82.

D-N-Acetylglycosamineanilide (XXIIa).—A mixture of 5.0 g. of N-acetylglycosamine,[23] 3.8 g. of aniline, 0.25 ml. of 2 *N* hydrochloric acid and 5 ml. of water was heated on the steam-bath for 15 minutes. The solution was neutralized with 0.5 ml. of 1 *N* sodium hydroxide and cooled. Two grams of needles of XXIIa was collected, washed with a small amount of cold water and recrystallized from ethanol; m.p. 206–207°, $[\alpha]^{24}D$ −69.0° (*c*, 1 in pyridine).

Anal. Calcd. for $C_{14}H_{20}N_2O_5$: C, 56.71; H, 6.81. Found: C, 56.43; H, 6.82.

XXIIa could not be hydrogenated over platinum in either neutral or alkaline solution. It gave a negative Benedict test but after short acid hydrolysis, the test was positive.

The triacetate of XXIIa was formed by the acetic anhydride–pyridine technique. Needles were obtained by recrystallization from alcohol; m.p. 181–182°, $[\alpha]^{24}D$ −63.5° (*c*, 1 in chloroform).

Anal. Calcd. for $C_{20}H_{26}N_2O_8$: N, 6.63; for $C_{22}H_{28}N_2O_9$: N, 6.03. Found: N, 6.68.

Sodium Metaperiodate Oxidation of XXIIa.—XXIIa (0.2168 g.) was dissolved in 10 ml. of hot water. After rapidly cooling, 17 ml. of 0.142 *M* sodium metaperiodate (3.28 molar equivalents) was added with rapid mixing. Aliquots withdrawn after one minute and ten minutes showed the consumption of 3.02 and 3.28 molar equivalents of oxidant.

To a solution of 0.192 g. of XXIIa in 25 ml. of water was added slowly over 15 minutes with rapid stirring, 25 ml. of an aqueous solution containing one molar equivalent of sodium metaperiodate. The reaction mixture was distilled into 25 ml. of an ethanol–water (1–4) solution of 0.200 g. of dimedon. After one hour 0.070 g. of the dimedon–formaldehyde compound was collected, m.p. 187–188° (38%).

***p*-N-Acetylglucosamine-*p*-nitroanilide (XXIIb).**—A mixture of 3.6 g. of N-acetylglucosamine, 4.5 g. of *p*-nitroaniline and 0.5 g. of ammonium chloride was refluxed for three days in 80 ml. of ethanol. During this time, the N-acetylglucosamine gradually dissolved. The ethanol was distilled *in vacuo* and the residue extracted three times with 50 ml. of hot benzene to remove unchanged *p*-nitroaniline. The residue was extracted with 50 ml. of boiling ethanol. On cooling 1.5 g. of the faintly yellow needles of XXIIb was obtained; m.p. 220° (dec.), $[\alpha]^{24}D$ −190° (*c*, 1 in pyridine).

Anal. Calcd. for $C_{14}H_{19}N_3O_7$: C, 49.24; H, 5.63; N, 12.31. Found: C, 49.06; H, 5.74; N, 12.85.

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