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Chloramphenicol[1] (Chloromycetin). VII. Synthesis through *p*-Nitroacetophenone

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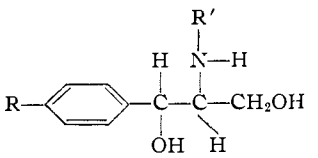
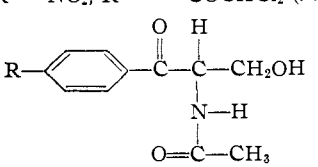
In the preceding paper[1a] of this series the authors discussed a synthesis of *dl-threo*-1-phenyl-2-amino-



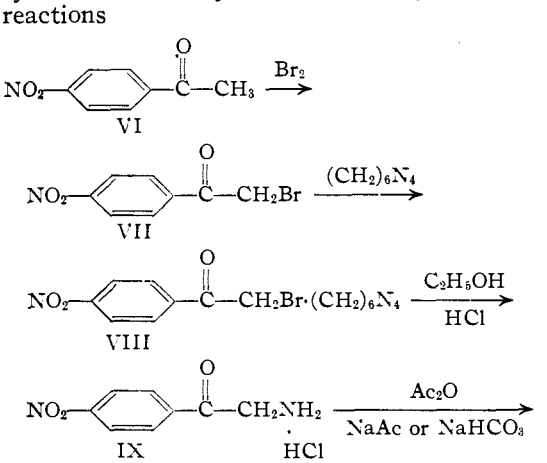
I, R and R′ = H
II, R = NO_2, R′ = H
III, R = NO_2, R′ = —$COCHCl_2$ (*l-threo*)

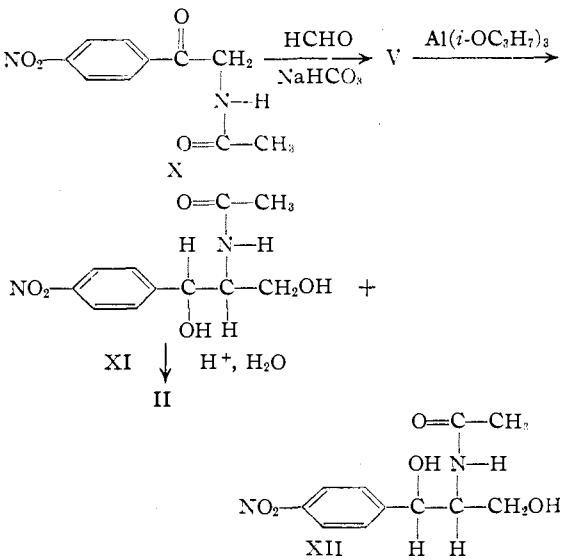


IV, R = H
V, R = NO_2

1,3-propanediol (I) and some of its derivatives. Nitration of the triacetyl derivative of I followed by hydrolysis produces II which may be resolved and the D-(−)-*threo*-isomer converted to chloramphenicol (III) by dichloroacetylation.[2]

Since the synthesis of I described by the authors[1] depends primarily upon the monohydroxymethylation of α-acetamidoacetophenone to form IV, the authors became interested in preparing *p*-nitro-α-acetamidoacetophenone in the expectation that this intermediate also could be monohydroxymethylated and the resulting propiophenone derivative (V) converted to II by reduction with an aluminum alkoxide. This synthesis of II may be illustrated by the series of reactions





p-Nitroacetophenone was prepared by the method of Walker and Hauser[3] with certain modifications. The ketone was brominated in practically quantitative yields[4] and the salt (VIII) with hexamethylenetetramine formed in chloroform or chlorobenzene. This salt has not been prepared previously although the *m*-nitro derivative is known.[5] Chloroform has been the solvent of choice[5,6] in the preparation of hexamethylenetetramine derivatives of phenacyl halides; however, for technical reasons, chloroform proved to be unsuitable for large scale preparations. Accordingly, several solvents were tried as substitutes for chloroform in the reaction. It was observed that chlorobenzene serves admirably even though the hexamethylenetetramine is less soluble in this solvent.

Hydrolysis of the salt to *p*-nitro-α-aminoacetophenone hydrochloride (IX) was accomplished with ethanol and concentrated hydrochloric acid. The attempted acetylation of IX with acetic anhydride in the presence of sodium hydroxide produced a dark-colored, tarry substance; but when sodium acetate or sodium bicarbonate was employed, excellent yields of *p*-nitro-α-acetamidoacetophenone (X) were obtained.

The monohydroxymethylation of X in the presence of sodium bicarbonate to form *p*-nitro-α-acetamino-β-hydroxypropiophenone (V) proceeded very satisfactorily in ethanol. The optimum temperature range seemed to be 35–40°. At temperatures up to 80° the desired product is

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

(2) Controulis, Rebstock and Crooks, *ibid.*, **71**, 2463 (1949).

(3) Walker and Hauser, *ibid.*, **68**, 1386 (1946).

(4) Engler and Zielke, *Ber.*, **22**, 204 (1889).

(5) Jacobs and Heidelberger, *J. Biol. Chem.*, **21**, 459 (1915).

(6) Mannich and Hahn, *Ber.*, **44**, 1542 (1911).

obtained but the yields are lower. The by-product is more water-soluble; and since an excess of formaldehyde is employed in the reaction, it is reasonable to suppose that more than one molecule of formaldehyde may react to form a polyhydroxy derivative. There is no tendency for V to form a hydrate as was the case with IV.[1]

The reduction of V with aluminum isopropoxide was performed under a variety of conditions. Whether the reaction was carried out at room temperature in a stream of nitrogen or at reflux temperature, the products were the same. Likewise, variation in the amount of aluminum isopropoxide influenced only the time required for completion of the reduction. Experience indicated that the reaction mixture could not be worked up by the usual procedure.[7] Addition of cold, dilute hydrochloric acid when the reduction was complete produced negligible amounts of precipitate. Therefore, the reduction mixture was hydrolyzed by the addition of water alone. Extraction of the aluminum hydroxide with isopropyl alcohol, concentration of the extracts and treatment of the residue with ethyl acetate produced good yields of *dl-threo*-1-*p*-nitrophenyl-2-acetamido-1,3-propanediol (XI).

It is an interesting fact that XI is obtained in very good yields while the yields of XII are much lower. Of course, the reduction with aluminum isopropylate of ketones capable of yielding more than one pair of isomers does not necessarily produce equal amounts of the possible isomers, *e. g.*, the reduction of benzil or benzoin with aluminum isopropylate yields 90% of *meso*-hydrobenzoin.[8]

The principal by-product is a thick liquid having properties similar to an oxazole or oxazoline. A second by-product has been isolated in low yields as a yellow crystalline substance which is under further investigation. Evidently, the β-hydroxy group of V is responsible for the additional reaction since the aluminum isopropoxide reduction of X gives good yields of 1-*p*-nitrophenyl-2-acetamidoethanol.

Experimental[9]

***p*-Nitroacetophenone (VI).**—The following procedure is similar to that of Walker and Hauser,[3] but differs in several important details.

Thirty-one and four-tenths grams (0.88 mole) of magnesium turnings and a solution of 4 ml. of dry carbon tetrachloride in 20 ml. of absolute ethanol were placed in a 2-l. flask equipped with a mechanical stirrer, a dropping funnel, a thermometer and a reflux condenser. As soon as the magnesium began to react with the ethanol, 200 ml. of dry chlorobenzene was added rapidly, the reaction between magnesium and ethanol continuing. A solution of 140.8 g. (0.88 mole) of diethyl malonate, 100 ml. of chlorobenzene and 80 ml. of absolute ethanol was added with stirring and cooling at such a rate as to keep the temperature at about 65°. When the reaction had proceeded to the extent that removal of the cooling bath did not result in a rise in temperature, the mixture was heated slowly to 85° (Glas-Col mantle) and kept there until the amount of unreacted magnesium was constant (about two hours).

The clear, dark solution was cooled to 25°, and a solution of 148.4 g. (0.8 mole) of *p*-nitrobenzoyl chloride in 500 ml. of dry chlorobenzene was added with stirring and moderate cooling so that the temperature did not exceed 35°. When about 80% of the acid chloride had been added, a brown, gelatinous mass precipitated and stirring became somewhat difficult. The mixture was stirred for thirty minutes at 35°. The flask was then cooled in an ice-bath and a solution of 50 ml. of concentrated sulfuric acid in 350 ml. of water was added immediately, slowly at first and then more rapidly.

The mixture was transferred to a separatory funnel and the lower layer, saturated with sodium sulfate, was discarded. The chlorobenzene layer was concentrated *in vacuo* (water-pump at 60–75°). The residue was refluxed with 240 ml. of glacial acetic acid, 30 ml. of concentrated sulfuric acid and 160 ml. of water for six hours.[10] The cooled hydrolysis mixture was added slowly with stirring to 1 l. of ice and water. The solid product was removed by filtration and washed with cold water. It was then melted under 800 ml. of water and stirred while 100 g. of sodium bicarbonate was added. The cooled mixture was filtered and the product was again treated with sodium bicarbonate (20 g.). The solid was filtered from the cooled mixture and recrystallized from 70 ml. of ethanol; yield of pale yellow crystalline product 98 g. (74%), m. p. 80°. Acidification of the sodium bicarbonate washings yielded 16 g. of *p*-nitrobenzoic acid.

Hexamethylenetetramine Salt of *p*-Nitro-α-bromoacetophenone (VIII).—Ninety-four grams (0.67 mole) of powdered hexamethylenetetramine was mixed with 570 ml. of dry chlorobenzene in a 2-l. flask equipped with an efficient stirrer and a thermometer. The mixture was stirred at room temperature for a few minutes and a solution of 149 g. (0.61 mole) of *p*-nitro-α-bromoacetophenone, prepared by bromination in acetic acid,[4] in 570 ml. of chlorobenzene was added in one portion with stirring. The temperature of the thick mixture slowly increased to 50–52° where it was maintained by external heating for four hours. The mixture was cooled to 30° and filtered. The filter-cake was stirred with 400 ml. of ethanol, filtered and washed with 100 ml. of ethanol. This process was repeated. The product was dried *in vacuo* over calcium chloride; yield 224.5 g. (95%), m. p. 118–120° (dec.).

***p*-Nitro-α-aminoacetophenone Hydrochloride (IX).**—The hexamethylenetetramine salt obtained from 48.8 g. (0.2 mole) of *p*-nitro-α-bromoacetophenone was added to a solution (25°) of 175 ml. of 95% ethanol and 85 ml. of concentrated hydrochloric acid in a 1-l. flask equipped with a stirrer and a thermometer. The resulting suspension was stirred at room temperature for sixteen hours. The mixture was cooled to 5° and filtered. The crystalline product, which consists of the amine hydrochloride and ammonium chloride, was stirred for fifteen minutes with 100 ml. of water (25°), cooled to 10° and filtered. The product was dried *in vacuo* over calcium chloride; yield 32 g. (74%, based on *p*-nitro-α-bromoacetophenone), m. p. 250° (dec.).

Anal. Calcd. for $C_8H_8N_2O_3 \cdot HCl$: N, 12.93. Found: N, 12.81, 12.84.

If the amine hydrochloride is refluxed with water, it decomposes and a tar is produced. However, recrystallization from water is quite successful provided a small excess of hydrochloric acid is present. Also, if the product is dried in air at 70° for several hours, decomposition occurs.

***p*-Nitro-α-acetamidoacetophenone (X).**—Thirty-two grams (0.148 mole) of *p*-nitro-α-aminoacetophenone hy-

(7) Wilds in "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 203.

(8) Wilds, *ibid.*, p. 185.

(9) The analytical data were determined by Mrs. Patricia Ramey and Mr. C. E. Childs of this Laboratory.

(10) Hydrolysis and decarboxylation may be effected in 20–40% sulfuric acid without a decrease in yield; but the presence of acetic acid in the hydrolysis of larger quantities of the ester is a definite advantage.

drochloride was mixed with 100 ml. of water and 200 g. of chipped ice in a 1-l. flask fitted with a stirrer, a thermometer and a dropping funnel. Thirty grams of acetic anhydride was added to the mixture which was then stirred while a solution of 40 g. of sodium acetate (trihydrate) in 150 ml. of water was added rapidly. The temperature of the mixture was kept below 10°, ice being added if necessary.

After the addition of the sodium acetate was complete the mixture was stirred until the temperature increased to 20°. It was then made strongly acid with hydrochloric acid and filtered. The pale yellow product was washed several times with water and dried; yield 30 g. (67%, based on *p*-nitro-α-bromoacetophenone), m. p. 157–160°. A small sample was recrystallized from ethyl acetate, m. p. 161–163°. The crystalline material develops a pink color when exposed to sunlight.

Anal. Calcd. for $C_{10}H_{10}N_2O_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.14; H, 4.72; N, 12.52.

p-Nitro-α-acetamido-β-hydroxypropiophenone (V).—A mixture of 44.4 g. (0.2 mole) of *p*-nitro-α-acetamidoacetophenone, 150 ml. of 95% ethanol and 33 ml. (*ca.* 0.4 mole) of 36–38% aqueous formaldehyde was prepared in a 500-ml. flask fitted with a stirrer and a thermometer. Two grams of sodium bicarbonate was added and the mixture was stirred for two hours at 35°. A clear solution was never formed, but the solid material changed crystalline form, becoming more dense. The mixture was cooled to 5° by the direct addition of Dry Ice and filtered. The solid product was washed with water, dilute hydrochloric acid and again with water and dried; yield 43.5 g. (86%), m. p. 164–166° (dec.). One gram was recrystallized from 30 ml. of absolute ethanol; yield 0.9 g., m. p. 166–167° (dec.).

Anal. Calcd. for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.62; H, 4.82; N, 11.23.

When the reaction was carried out using four equivalents of formaldehyde, a clear, red-colored solution was obtained after stirring and heating at 35° for about fifteen minutes, and within two to five minutes the product precipitated. By this procedure, using either methanol or ethanol as a solvent, the yields varied from 80 to 90%. However, if the *p*-nitro-α-acetamidoacetophenone was impure, the larger excess of formaldehyde gave lower yields of the monohydroxymethylated product.

***dl-threo*-1-*p*-Nitrophenyl-2-acetamido-1,3-propanediol (XI).**—Fifty and four-tenths grams (0.2 mole) of *p*-nitroacetamido-β-hydroxypropiophenone was added to a hot solution of 61.2 g. (0.3 mole) of aluminum isopropoxide (Edwal Laboratories, Inc.) in 500 ml. of dry isopropyl alcohol in a 1-l. flask equipped with a stirrer and a Hahn condenser.[11] After a few minutes an opaque, red-brown solution was formed. Stirring and removal of acetone was continued for seven hours, at which time the acetone test was negative. During the last half-hour, distillation was quite rapid so that a total of 300 ml. of isopropyl alcohol was removed during the reaction.

The thick, red-brown residue was allowed to cool to just below the reflux temperature before 50 ml. of water was added. The mixture was refluxed for fifteen minutes and filtered. During the refluxing the color changed to light yellow. The residue was treated twice by refluxing with additional 250-ml. portions of 80% isopropyl alcohol. The combined extracts were concentrated *in vacuo*. The residue was refluxed with 100 ml. of ethyl acetate until the oily material had dissolved, cooled to 20° and filtered. The crystalline product so obtained was washed with ethyl acetate and dried; yield 25 g., m. p. 159–164°. Recrystallization was accomplished by solution in 80 ml. of boiling water and cooling; yield 21 g. (41%, m. p. 167–169°.

Anal. Calcd. for $C_{11}H_{14}N_2O_5$: C, 51.96; H, 5.55 Found: C, 52.12; H, 5.51.

By allowing the aqueous filtrate from the recrystallization above to stand for several days, 2 g. of the *dl-erythro*-racemate, m. p. 195°, was obtained. The residue from the ethyl acetate filtrate is under investigation at present.

***dl-threo*-1-*p*-Nitrophenyl-2-amino-1,3-propanediol (II).**—A mixture of 25.4 g. (0.1 mole) of the monoacetyl derivative (XI) and 254 ml. of 5% hydrochloric acid was heated on a steam-bath with occasional swirling. The solid dissolved within a few minutes and the resulting solution was heated for one hour (95°). The solution was cooled slightly, mixed with a moderate amount of charcoal and filtered. The pale yellow solution was cooled to 20° and made strongly basic with 20% aqueous sodium hydroxide. On cooling the solution, an almost colorless solid precipitated. Scratching of the walls of the flask may be necessary to induce precipitation. The product was filtered off and recrystallized from water; yield 18 g. (85%), m. p. 143–145°.

Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70. Found: C, 50.87; H, 5.58.

1-*p*-Nitrophenyl-2-acetamidoethanol.—Four grams (0.018 mole) of *p*-nitro-α-acetamidoacetophenone was reduced with 5.5 g. (0.027 mole) of aluminum isopropoxide[12] and the product isolated as above. After recrystallization from ethyl acetate and from water the yield was 2.8 g. (69%), m. p. 152–154°.

Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.52; H, 5.40; N, 12.55. Found: C, 53.49; H, 5.61; N, 12.50.

Summary

A synthesis of chloramphenicol starting with *p*-nitroacetophenone has been carried through the essential steps. Optimum conditions for the monohydroxymethylation of *p*-nitro-α-acetamidoacetophenone are the presence of a small amount of a weakly alkaline substance such as sodium bicarbonate and an excess of formaldehyde at 35–40°.

Reduction of *p*-nitro-α-acetamido-β-hydroxypropiophenone with aluminum isopropoxide gives a good yield of the *dl-threo*-racemate from which the chloramphenicol intermediate aminediol may be obtained by resolution. The *dl-erythro*-racemate is recovered in a much lower yield.

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(11) Wilds in "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 197.

(12) The amide group is apparently responsible for the production of the red-brown color, since the color mentioned in the reduction of XI was also observed here; whereas the reduction of *p*-nitroacetophenone in our laboratories produced only a slight color.