

Photodegradation Products of Chloramphenicol in Aqueous Solution

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Abstract □ An aqueous solution of chloramphenicol was degraded upon exposure to sunlight, UV, and tungsten light. The major photodegradation products were hydrochloric acid, *p*-nitrobenzaldehyde, *p*-nitrobenzoic acid, 4,4'-azoxybenzoic acid, and *p*-aminophenyl-2-acetamido-1,3-propanediol. Photolysis of *p*-nitrobenzaldehyde in water gave exclusively one product, *p*-nitrosobenzoic acid, with 92% yield. The data obtained suggest that *p*-nitrosobenzoic acid may be an intermediate in the reaction mixture of chloramphenicol. Photolysis of chloramphenicol in ethanol and benzene was also similarly conducted. No degradation products were found. The chemistry of the photodegradation products suggests that chloramphenicol in water under the influence of light undergoes oxidation, reduction, and condensation reactions.

Keyphrases □ Chloramphenicol—six photodegradation products in aqueous solution isolated and identified □ Photodegradation products, chloramphenicol—isolated and identified, from water, ethanol, and benzene, using sunlight, UV, and tungsten light □ Light stability, chloramphenicol—using sunlight, UV, and tungsten light, in three solvents, six photodegradation products isolated and identified

The effect of light is often considered an important factor in drug stability. Nevertheless, there is only little information on the light stability of chloramphenicol (1). Photodegradation reactions of this antibiotic are practically unknown. The present study was undertaken to isolate and identify the major photolysis products of chloramphenicol. Experiments were conducted in solvents of different polarity (*i.e.*, water, ethanol, and benzene) using sunlight, UV, and tungsten light. Six photodegradation products are reported.

EXPERIMENTAL¹

Materials—Phenylhydrazine hydrochloride, *p*-nitrobenzaldehyde, *p*-nitrobenzoic acid, *N*-(1-naphthyl)ethylenediamine dihydrochloride, sodium nitrite, ammonium sulfamate, stannous chloride, zinc dust, aluminum oxide, and solvents were reagent grade (Fisher). Eastman chromatogram sheet (6061, silica gel) was used. The reference material of chloramphenicol used in this study was white, crystalline chloramphenicol of high purity², m.p. 151° [lit. (2) m.p. 150–153°].

Photolysis of Chloramphenicol in Aqueous Solution—One liter of the sample solution (0.25%, pH 5.4) was prepared with distilled water in a stoppered glass flask and exposed to sunlight in a temperature range of 21–30°. As photolysis progressed, the sample solution slowly turned yellow, and orange-yellow precipitates were observed to form. After 96 hr. of irradiation, the solution was filtered (pH 1.9). The filtrate was designated as Fraction A, and the crude product was designated as Fraction B (yield 404 mg.). Sample solutions were also irradiated with UV and tungsten light in an air-conditioned room. The distance between light sources and samples was approximately 5 cm. The temperature of the sample solutions reached an equilibrium after 4 hr., *i.e.*, 28–29° (UV) and 39–40°

(tungsten light). After 96 hr. of irradiation, the samples were analyzed. Major photodegradation products were found to be essentially the same as those identified from sunlight-treated sample. Control samples (0.25%) were prepared in low actinic flasks and treated in the same way as the samples. No degradation products were detected.

Photolysis of Chloramphenicol in Ethanol and Benzene—Samples were dissolved in ethanol and benzene (0.25%). Solutions were irradiated in the same way as previously described. No degradation products were detected.

Isolation and Identification of Major Components from Fraction A—Hydrochloric Acid (Acidimetric and Volhard's Chloride Determination)—Fifty milliliters of Fraction A was titrated with 0.1 *N* sodium hydroxide solution, using phenolphthalein T.S. as the indicator. A freshly prepared sample solution (0.25%) served as the blank. After correction for the blank, 6.00 ml. of titrant was consumed. The total yield of hydrogen-ion concentration in 1 l. of sample solution was thus found to be 12 milliequivalents. Fifty milliliters of Fraction A also was titrated with 0.05 *N* silver nitrate solution. After correction for the blank, 12.05 ml. of titrant was consumed. The total yield of chloride-ion concentration in 1 l. of sample solution was thus found to be 12.05 milliequivalents.

***p*-Nitrobenzaldehyde**—Two hundred milliliters of Fraction A was distilled. Approximately 150 ml. of distillate was collected and then extracted with two 50-ml. portions of ether. Evaporation of the ether extract yielded 33 mg. of product. The product was recrystallized from hot water, m.p. 106° (uncorr.) [lit. (3) m.p. 107°]. No depression of melting point with an authentic sample of *p*-nitrobenzaldehyde was observed. The IR spectrum (KBr) of the product was found to be essentially the same as that of *p*-nitrobenzaldehyde.

***p*-Nitrobenzoic Acid**—Two hundred milliliters of Fraction A was made alkaline with 5 ml. of sodium hydroxide solution (10% w/v) and extracted with six 60-ml. portions of ether. The aqueous phase was then acidified with 10 ml. of dilute hydrochloric acid and extracted with two 50-ml. portions of ether. Evaporation of the ether extract yielded about 24 mg. of crude product. Sublimation at a temperature range of 210–220° under atmospheric pressure yielded a pure substance, m.p. 240° (uncorr.) [lit. (3) m.p. 241°]. No melting-point depression of the product with an authentic sample of *p*-nitrobenzoic acid was observed. The IR spectrum (KBr) of the product was found to be essentially the same as that of *p*-nitrobenzoic acid.

***p*-Aminophenyl-2-acetamido-1,3-propanediol**—One hundred milliliters of chloramphenicol solution (0.25%) was mixed for 6 hr. with 0.15 g. of zinc dust at room temperature. The reaction mixture was then filtered. A clear filtrate was obtained which, on standing, turned yellow, exhibiting essentially the same UV spectrum as previously reported (4), *i.e.*, λ_{\max} . 237 nm. and λ_{\min} . 217 nm. This compound was not isolated and was assumed to be *p*-aminophenyl-2-acetamido-1,3-propanediol (4).

Approximately 20–30 μ l. of Fraction A was spotted on a thin-layer plate along with 5 μ l. (containing about 1 mcg.) of reference sample, which was synthesized as described previously. The thin-layer plate was developed in a solvent system of two parts methanol and one part chloroform. After development, the plate was sprayed consecutively with 0.1% sodium nitrite, 0.2% hydrochloric acid, 0.4% ammonium sulfamate, and 0.2% *N*-(1-naphthyl)ethylenediamine dihydrochloride aqueous solutions. One spot from Fraction A corresponded to the reference sample and showed a purple to pink color, R_f 0.75.

Isolation and Identification of Major Components from Fraction B—4,4'-Azoxybenzoic Acid—A sample of 150 mg. of Fraction B was dissolved in 5 ml. of ammonium hydroxide T.S. The sample solution was then transferred to an aluminum oxide column (2.4 × 64 cm.) packed with a slurry of aluminum oxide, 120 g. in methanol. The column was eluted with about 100 ml. of methanol and then

¹ The following equipment was used: Bausch & Lomb recording spectrophotometer, 505; Beckman IR-8; UV lamp (emitting light mainly at 365 nm., Fisher); and tungsten light (ordinary light bulb, 100 w.).

² Obtained from Parke-Davis.

with 50% methanol. Approximately 200 ml. of pale-yellow eluate was collected. Acidification of the eluate with glacial acetic acid gave a pale-yellow product (yield 120 mg.).

Purity of the product was tested as follows. Four milligrams of the sample was dissolved in 10 ml. of 0.1 *N* sodium hydroxide solution. Then 5 μ l. of the sample solution was spotted on a thin-layer plate and developed in a solvent system of 10 parts methanol and 0.1 part glacial acetic acid. After development, the plate showed only one distinct spot when visualized under visible light or UV lamp, R_f 0.67.

The product was insoluble in alcohol, ether, and most organic solvents but was readily soluble in organic bases, sodium bicarbonate, and alkaline solution; m.p. about 240° dec.; UV spectrum: $a = 62.5$, λ_{\max} 333 nm. (0.1 *N* sodium hydroxide); IR spectrum: $\bar{\nu}_{\max}^{\text{KBr}}$ 2648, 2538, 1672, 1592, 1433, 1416, 1284 cm^{-1} , etc.; mass spectrum (isotopic analysis)—Calc. for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_5$: m/e 286 (100), 287 (16.3), 288 (2.2). Found: m/e 286 (100), 287 (16.5), 288 (2.2). The neutralization equivalent was: 144 (found), 143 (theory).

Anal.—Calc. for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_5$: C, 58.74; H, 3.49; N, 9.77. Found: C, 58.65; H, 3.50; N, 9.90.

Synthesis of 4,4'-Azoxybenzoic Acid—One gram of *p*-nitrobenzoic acid was dissolved in a mixture of 100 ml. ethanol and 10 ml. glacial acetic acid. Then 0.58 g. zinc dust was slowly added. The reaction mixture was gently refluxed for 1 hr. The product was filtered and washed with hot methanol (yield 0.2 g.).

The purity of the product was tested by using the same TLC technique as previously described. Only one spot was observed when visualized under visible or UV lamp, R_f 0.67; m.p. about 240° dec.; UV spectrum: $a = 62.9$, λ_{\max} 333 nm. (0.1 *N* sodium hydroxide). The IR spectrum (KBr) of this synthetic substance was identical to that of the product isolated from the chloramphenicol irradiation study. The neutralization equivalent was: 141 (found), 143 (theory).

Anal.—Calc. for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_5$: C, 58.74; H, 3.49; N, 9.77. Found: C, 58.52; H, 3.64; N, 9.62.

p-Nitrosobenzoic Acid—Two grams of *p*-nitrobenzaldehyde was dissolved in 2 l. of hot water. The sample solution was then exposed to sunlight (21–30°) for 96 hr. The product was filtered and washed with hot water until the washings gave a negative test with phenylhydrazine T.S. A pale-yellow product was obtained (yield 1.84 g., 92% of theory).

The purity of the product was tested as follows. A sample of 4 mg. was dissolved in 10 ml. of 0.1 *N* sodium hydroxide solution. Then 5 μ l. was spotted on a thin-layer plate and developed in a solvent system of 20 parts chloroform and 0.1 part glacial acetic acid. The plate showed only one distinct spot under a UV lamp, R_f 0.30. After spraying the plate consecutively with 0.1% stannous chloride (prepared in dilute hydrochloric acid), 0.1% sodium nitrite, 0.4% ammonium sulfamate, and 0.2% *N*-(1-naphthyl)-ethylenediamine dihydrochloride aqueous solutions, a purple-to-pink spot was developed. This color reaction is typical of aromatic nitro compounds. To differentiate this compound from *p*-nitrobenzoic acid, a specific color spot test was developed. After spraying the thin-layer plate with a 0.2% alkaline 2-naphthol solution (prepared in sodium hydroxide T.S.), a gray color spot was developed which, on standing, slowly turned pink. The limit of this test was about 0.5 mcg., and *p*-nitrobenzoic acid failed to give this test.

The product was not soluble in water or most organic solvents but was readily soluble in organic bases, sodium bicarbonate, and alkaline solutions; m.p. about 250° dec.; UV spectrum: $a = 56.6$, λ_{\max} 288 nm. and $a = 63.6$, λ_{\max} 313 nm. (0.1 *N* sodium hydroxide); IR spectrum (KBr): $\bar{\nu}_{\max}$ 2646, 2538, 1678, 1592, 1422, 1292, 1263 cm^{-1} , etc.; NMR spectrum (solvent dimethyl sulfoxide), $J = 9$ Hz. (coupling constant of *ortho*-hydrogen). The neutralization equivalent was: 149 (found), 151 (theory).

Anal.—Calc. for $\text{C}_7\text{H}_5\text{NO}_3$: C, 55.60; H, 3.31; N, 9.27. Found: C, 54.99; H, 3.30; N, 9.42.

This compound was not detected in the photolysis mixture of chloramphenicol.

DISCUSSION

The photolysis of chloramphenicol was conducted in solvents of different polarities, *i.e.*, water, ethanol, and benzene. Sample

solutions were exposed to sunlight, UV, and tungsten light. Control samples were prepared in low actinic flasks and treated in the same way as the samples. After 96 hr. of irradiation, samples were analyzed. Degradation products were present only in aqueous sample solutions. No products were detected either in control samples or samples prepared in ethanol and benzene. The evidence thus suggested that the degradation products, as subsequently described, were not present in the starting material of chloramphenicol but were produced as a result of a photochemical reaction. Preliminary tests, originally designed for other studies, indicated that chloramphenicol in hydrochloric acid (0.1 *N*), when heated in an oven at 97° for 72 hr., did not yield any products other than normal hydrolyzates. It is, therefore, concluded that neither temperature nor high hydrogen-ion concentration would cause the observed photochemical reactions. The products described herein must be the photodegradation products.

The major photodegradation products of chloramphenicol were isolated and identified. They were *p*-nitrobenzaldehyde, *p*-nitrobenzoic acid, 4,4'-azoxybenzoic acid, and *p*-aminophenyl-2-acetamido-1,3-propanediol. Since one or two acids in addition to hydrochloric acid are present in the photolysis mixture of chloramphenicol, results obtained from hydrogen- and chloride-ion determination merely support the possibility that hydrochloric acid is a product of the reaction.

p-Nitrobenzaldehyde was detected after a few minutes of irradiation of an aqueous solution of chloramphenicol. For this reason, photolysis of *p*-nitrobenzaldehyde was performed. Sunlight irradiation of an aqueous *p*-nitrobenzaldehyde solution gave exclusively *p*-nitrosobenzoic acid with 92% yield. To distinguish this compound from *p*-nitrobenzoic acid, a specific color spot test using alkaline 2-naphthol solution was developed.

Condensation between nitroso and hydroxyamino compounds is a well-known reaction, giving rise to the azoxy compound (5). Although this reaction was facilitated in an alkaline condition, condensation may also proceed in an acid medium, as exemplified by the chemical synthesis of 4,4'-azoxybenzoic acid employed in this study. *p*-Nitrosobenzoic acid was not detected in the photolysis mixture of chloramphenicol. However, subsequent isolation and identification of 4,4'-azoxybenzoic acid provided indirect evidence for the possible intermediate formation of *p*-nitrosobenzoic acid in the irradiated chloramphenicol reaction system, although the photochemical mechanism was not ascertained in the present study.

Chemistry of the photodegradation products suggests that chloramphenicol in water under the influence of light undergoes oxidation, reduction, and condensation reactions.

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