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Degradation Products of Chloramphenicol

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Abstract □ Incubated aqueous solutions of chloramphenicol at various pH's (1–14) yielded detectable amounts of *p*-nitrobenzaldehyde (an oxidation product) and arylamine (a reduction product). Identical degradation products were also found in certain dosage forms (creams and capsules), although they were not found in ophthalmic ointment.

Keyphrases □ Chloramphenicol—degradation products determined □ *p*-Nitrobenzaldehyde—determined as degradation product in chloramphenicol □ Arylamine—determined as degradation product in chloramphenicol

The stability of chloramphenicol is known to be dependent in part upon two degradation pathways: amide hydrolysis at pH 2–7 and carbon–chlorine cleavage about pH 7 (1, 2). Amide hydrolysis leads to the formation of *p*-nitrophenyl-2-amino-1,3-propanediol. This compound was detected in dosage forms by Sahli *et al.* (3), Kassem *et al.* (4), Rousselet and Paris (5), and James and Leach (6). Other degradation products have not been reported in dosage forms of chloramphenicol below pH 7.0.

As suggested by Higuchi and Bias (2), the reactivity of chloramphenicol could lead to other degradation reactions. Initial studies in these laboratories revealed that aged chloramphenicol preparations contained arylamine and *p*-nitrobenzaldehyde. The present study was designed to illustrate the identification of these degradation products and to measure the concentrations of *p*-nitrobenzaldehyde in various chloramphenicol preparations.

EXPERIMENTAL

Materials and Apparatus—Reference standard chloramphenicol was used[1]. All other chemicals and solvents were of reagent grade. Silica gel sheets (chromatogram sheets, Eastman No. 6061) were used for TLC. The chromatographic solvent systems are shown in Table I. Spectrophotometric analyses were performed with the Bausch & Lomb 505 (visible) and the Beckman IR-8 (IR).

Spontaneous Oxidation and Reduction of Chloramphenicol in Aqueous Solution—Fourteen aqueous solutions (0.2%) of chloramphenicol were prepared in low actinic volumetric flasks ranging from pH 1 to 14, respectively. After standing at room temperature for 24 days or longer, 50 ml. of each sample solution was extracted with approximately 25 ml. ether. Evaporation of the ether extract yielded a residue. Tests were then performed for the presence of oxidation and reduction products by using the same methods as subsequently described for chloramphenicol dosage forms.

Identification of *p*-Nitrobenzaldehyde and Its Occurrence in Dosage Forms—The contents of 10 capsules, equivalent to 2.5 g. of chloramphenicol, were stirred with 400 ml. water for 0.5 hr. The suspension was filtered and the filtrate distilled. Approximately 300 ml. of distillate was collected and extracted with 50 ml. ether. Evaporation of the ether extract yielded a small amount of residue possessing the characteristic odor of *p*-nitrobenzaldehyde. The residue, Product A, was dissolved in 0.5 ml. methanol; 10–30 μl. was spotted on a thin-layer plate along with 5 μl. (containing 2 mcg.) of the reference *p*-nitrobenzaldehyde. After development, the TLC plates were sprayed with 0.5% alcoholic phenylhydrazine solution. A similar procedure was followed for chloramphenicol cream and ophthalmic ointment, except that a 20-g. sample was taken and a small amount of antifoam was introduced in the distillation step.

An alternate procedure was used as follows: the contents of five capsules were extracted with approximately 50 ml. ether and filtered. To the filtrate, a few drops of 0.5% alcoholic phenylhydrazine solution were added and the solution was evaporated. The orange-yellow phenylhydrazine derivative was extracted with 0.5 ml. of solvent mixture (two parts CCl_4 and one part $CHCl_3$). Then 10–30 μl. of this solution (Solution B) was spotted on a thin-layer plate along with 5 μl. (containing 2 mcg.) of a reference sample of *p*-nitrobenzaldehyde phenylhydrazone.

IR Identification of *p*-Nitrobenzaldehyde as Its Phenylhydrazone Derivative—The phenylhydrazone derivative was obtained as described previously. The orange-yellow spots of phenylhydrazone were cut out and extracted with methanol. Evaporation of methanol yielded a small amount of dark-orange residue, which was triturated with a small amount of potassium bromide. The IR spectrum of the sample in KBr pellets was compared to that of an authentic sample of *p*-nitrobenzaldehyde phenylhydrazone.

Estimation of *p*-Nitrobenzaldehyde in Chloramphenicol Dosage Forms—From the contents of 20 capsules, the powder equivalent to 1.0 g. of chloramphenicol, accurately weighed, was transferred to a 10-ml. volumetric flask and made to volume with ethanol. A filtered 5-ml. aliquot was mixed with 2 ml. of 0.5% alcoholic phenylhydrazine solution. After standing for 30 min., the absorbances of both the sample and standard solutions were measured against a reagent blank at 440 nm. A similar procedure was followed for chloramphenicol cream, except that a 5-g. sample was completely dissolved in an appropriate amount of ethanol and the cream base was separated by freezing. This ethanol extract was then treated as for capsules. The results are reported in Table II.

Identification of *p*-Nitrophenyl-2-amino-1,3-propanediol—The contents of four capsules were mixed in 40 ml. methanol and filtered. The filtrate was evaporated and the residue extracted with 2–3 ml. water. Then 30–60 μl. of the sample preparation (Solution C) was spotted along with 5 μl. (containing 10 mcg.) of reference *p*-nitrophenyl-2-amino-1,3-propanediol on a thin-layer plate. After development, the plate was sprayed with 0.2% alcoholic ninhydrin solution and heated in an oven for about 5 min. at 60°. For creams, a 5-g. sample was melted on a water bath, and 20 ml. of 95% ethanol was added. Most of the cream base separated on freezing, and the mixture was filtered through a cotton pad. The filtrate was evaporated to dryness, and the residue was treated as for capsules. A

[1] Obtained from Parke, Davis and Co.

Table I—R_f Values for Reference Materials and Degradation Products[a]

Compound	$CHCl_3$–CCl_4 (1:1)	$CHCl_3$–CCl_4 (2:1)	$CHCl_3$–CCl_4 (1:2)	CH_3OH–$CHCl_3$ (2:1)	CH_3OH–$CHCl_3$ (1:1)
p-Nitrobenzaldehyde reference	—	—	0.61	—	—
Degradation Product A	—	—	0.61	—	—
p-Nitrobenzaldehyde phenylhydrazone reference	0.56	0.69	0.17	—	—
Degradation Product B as phenylhydrazone derivative	0.56	0.69	0.17	—	—
p-Nitrophenyl-2-amino-1,3-propanediol reference	—	—	—	0.58	—
Degradation Product C	—	—	—	0.58	—
Arylamine reference	—	—	—	—	0.75
Degradation Product D	—	—	—	—	0.75

[a] No degradation products were found in expired ophthalmic ointments.

similar procedure was used for an ophthalmic ointment. The R_f values are reported in Table I.

Identification of Arylamine in Chloramphenicol Dosage Forms—Contents of five capsules were extracted with approximately 20 ml. water. The aqueous extract was shaken with about 40 ml. ether. Evaporation of the ether extract yielded a residue, to which was added 0.2 ml. water (Solution D). Then 40–60 μl. was spotted on a thin-layer plate along with 5 μl. of reference arylamine (containing about 1 mcg.), which was synthesized by zinc dust reduction of chloramphenicol in distilled water. After development, the thin-layer plates were sprayed consecutively with 0.1% sodium nitrite, 0.2% HCl, 0.4% ammonium sulfamate, and 0.2% *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution. A similar procedure was used for an ophthalmic ointment. As for the chloramphenicol cream, 3–5-g. samples were mixed slowly with about 30 ml. water to avoid emulsification. The solution was siphoned through cotton. Then 10–15 ml. of the clear solution was collected and extracted with about 20 ml. ether. Evaporation of ether extract yielded a residue, which was treated in the same manner as with capsules.

DISCUSSION

The experimental results (Table I) show clearly that both *p*-nitrobenzaldehyde and arylamine were present in certain chloramphenicol dosage forms which had been stored under normal conditions. *p*-Nitrophenyl-2-amino-1,3-propanediol was also found in this study, as had been previously reported. Expired water-free preparations such as ophthalmic ointment did not show the presence of any degradation product. This was in agreement with the results of James and Leach (6).

Aqueous solutions of chloramphenicol, ranging from pH 1 to 14, after standing at room temperature over a period of 24 days or longer yielded detectable amounts of *p*-nitrobenzaldehyde (an oxidation product) and arylamine (a reduction product), while freshly prepared solutions failed to show the presence of these products. Experimental findings thus give strong evidence to the effect of water on this new degradation pathway of chloramphenicol, which may well account for the presence of these two specific degradation products as found in certain water- or moisture-containing chloramphenicol dosage forms, *e.g.*, creams and capsules.

Table II—Quantitative Estimation of *p*-Nitrobenzaldehyde in Chloramphenicol Preparations

Sample	Manufacturers	*p*-Nitrobenzaldehyde, %[a]
Capsule	A	0.040
Capsule[b]	B	0.024
Capsule[b]	C	0.016
Cream[c]	C	0.090
Cream[d]	C	0.572

[a] Percentage of *p*-nitrobenzaldehyde was calculated on the basis of label claim of chloramphenicol. [b] Capsules of B and C were expired samples. [c] Label claim for chloramphenicol cream was 1%. [d] Cream was expired sample of 1 year.

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