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# Synthesis and Antibiotic Properties of Chloramphenicol Reduction Products

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Analogs of chloramphenicol were prepared for the first time in which the nitro group was replaced by hydroxylamine, nitroso, hydroxamic acid, methyl hydroxamate, and *O*-acetyl hydroxamate functional groups. These compounds were tested for antibiotic activity in order to determine whether the antibiotic activity of chloramphenicol is mediated by one or more of these potential metabolites of chloramphenicol. None of these analogs was as active as chloramphenicol against the four test organisms, and two of the compounds were essentially devoid of activity. The significance of these findings with regard to the importance of the nitro group to the biological activity of chloramphenicol is discussed.

Although chloramphenicol (I) is one of the oldest and most potent of the antibiotics used in chemotherapy, we still know relatively little about its precise mechanism of action, and even less about the mechanisms responsible for its toxic side effects (17, 28). Chloramphenicol is the leading cause of drug-induced aplastic anemia, yet little progress has been made in an attempt to explain the events leading to this toxic manifestation (17, 28). Our interest in the biochemical mechanisms of carcinogenesis by aromatic nitrogen compounds prompted us to undertake parallel studies on chloramphenicol. The analogy between the bone marrow necrosis associated with aplastic anemia and that leading to the induction of malignancies suggested to us that similar mechanisms might be operating in these two disease processes. A search of the scientific literature showed that our hypothesis had been made by Weisburger and co-workers 10 years earlier (25). Although this group was not the first to speculate that chloramphenicol side effects might be due to the production of toxic metabolites (29), they were the first to propose that such metabolites might be intermediate metabolic reduction products of the nitro moiety (25). This hypothesis is especially attractive in light of the fact that thiamphenicol, a potent chloramphenicol analog lacking the nitro group, has never been reported to cause aplastic anemia in spite of its extensive use (17, 28). It is most surprising that none of these reduction intermediates of chloramphenicol has ever been reported in the literature.

Numerous analogs of chloramphenicol have been prepared in an attempt to understand the structure-activity relationships of this antibiotic (9, 10). Attempts have also been made to produce a chloramphenicol derivative that is a selective inhibitor of bacterial protein synthesis and does not cause inhibition of mitochondrial protein synthesis (6, 17). We now report the preparation and antibiotic properties for a series of chloramphenicol analogs derived by partial reduction of the nitro group. Although none of these analogs possesses appreciable antibiotic activity, studies on their toxicological properties might prove to be very informative in view of related studies that have been reported (2, 15). Such studies are necessary since there is considerable literature precedence to suggest that the nitroso (III) and hydroxylamine (II) analogs would be expected metabolites produced by the nitroreductase activities of gastrointestinal microflora (19, 22, 27).

(A portion of this work was taken from the MS thesis of B.R.C., submitted to the University of Mississippi School of Pharmacy.)

## MATERIALS AND METHODS

Chloramphenicol was obtained from Sigma Chemical Co. (St. Louis, Mo). Silica gel for column chromatography and thin-layer chromatography (TLC) was EM brand, obtained from Brinkmann Co. (Westbury, N.Y.). Unless otherwise specified, all chemicals and solvents were of analytical reagent-grade purity. Mass spectra were obtained on a DuPont 21-492 high-resolution mass spectrometer. Sixty-megahertz nuclear magnetic resonance (NMR) spectra were obtained on a Jeolco C-60HL spectrometer, and 220-MHz spectra were performed by the Morgan-Schaffer Co. (Montreal, Quebec). Infrared (IR) spectra were obtained on a Perkin-Elmer 257 spectrophotometer, and ultraviolet (UV) spectra were obtained on a Beckman 24 spectrophotometer. Melting points were obtained on a calibrated Thomas Hoover melting point apparatus. Elemental analyses were performed by Gal-

braith Laboratories (Knoxville, Tenn.). Schemes for preparing the analogs are shown in Fig. 1.

**D(−)-*threo*-2-Dichloroacetamido-1-*p*-hydroxylaminophenyl-1,3-propanediol (II).** Chloramphenicol (I, 3.23 g, 0.01 mol) and $NH_4Cl$ (1 g, 0.19 mol) were stirred vigorously in 40 ml of 25% EtOH while Zn dust (2.6 g, 0.04 g-atom) was added in small portions during the course of 10 min. The mixture was stirred for an additional 15 min, after which 10 ml of water was added and the suspension was filtered. The filter cake was washed with 20 ml of EtOAc; the filtrate was then saturated with NaCl and extracted three times with 50-ml portions of EtOAc. The combined EtOAc extracts were washed with 20 ml of water, dried ($Na_2SO_4$), and evaporated in vacuo to give impure II as a yellow oil (2.9 g, 93%), which was generally used in subsequent reactions without further purification. For purification as required for characterization and antibiotic testing, the oil was chromatographed on a column (2 by 60 cm) of silica gel (80 g), using stepwise elution from 25% $MeOH/CH_2Cl_2$ to 50% $MeOH/CH_2Cl_2$. Fractions containing only II, as determined by TLC analysis using 5% aqueous pentacyanoamine ferroate spray reagent for visualization (21), were combined and evaporated to give II as a yellow oil (1.5 g, 48%). IR (film) 3,300 $cm^{-1}$ (OH, NHOH), 1,690 $cm^{-1}$ (C=O); NMR ($CD_3OD$) $\delta$ 6.97 (sym. multiple, 4H, $C_6H_4$), $\delta$ 6.03 (single, 1H, $CHCl_2$), $\delta$ 4.88 (double, 1H, $C_6H_4$-CH), $\delta$ 4.03 (multiple, 1H, NH-CH), $\delta$ 3.61 (multiple, 2H, $CH_2OH$).

**D(−)-*threo*-2-Dichloroacetamido-1-*p*-nitrosophenyl-1,3-propanediol (III).** Chloramphenicol (I, 3.23 g, 0.01 mol) and $NH_4Cl$ (1 g, 0.019 mol) were stirred vigorously in 40 ml of 25% EtOH while Zn dust (2.6 g, 0.04 g-atom) was added in small portions over 15 min. The mixture was stirred for an additional 15 min, after which 10 ml of water was added and the suspension was filtered. The filter cake was washed with 15 ml of water, and the filtrate was extracted three times with 50-ml portions of $Et_2O$. TLC analysis showed that unreacted I was in the $Et_2O$ portion and that the hydroxylamine (II) was concentrated in the water portion. The aqueous solution of the hydroxylamine was chilled and poured, with stirring, into an ice-cold solution of $FeCl_3 \cdot 6H_2O$ (6.6 g, 0.024 mol) in 50 ml of water. The solution immediately turned green; it was stirred for an additional 30 min at 0°C and then extracted three times with 50-ml portions of $Et_2O$. The $Et_2O$ solution was dried ($Na_2SO_4$) and evaporated to give a blue-green residue. The residue was chromatographed on silica gel (70 g) with 5% $MeOH/CHCl_3$ as eluent. The blue band which eluted was collected, and the solvent was removed in vacuo to give a pale blue solid, which recrystallized slowly from dichloroethane to give III as blue needles (0.94 g, 30%). mp 129.5 to 130.5°C; $UV_{max}^{EtOH}$ 292 nm (log $\epsilon$ 3.9) and 318 nm (log $\epsilon$ 4.0); IR (KBr) 3,400 $cm^{-1}$ (OH), 3,240 $cm^{-1}$ (NH), 1,670 $cm^{-1}$ (C=O), 1,550 $cm^{-1}$ (N=O); NMR ($CD_3OD$) $\delta$ 7.83 (sym. multiple, 4H, $C_6H_4$), $\delta$ 6.27 (single, 1H, $CHCl_2$), $\delta$ 5.20 (double, 1H, $C_6H_4$-CH), $\delta$ 4.65 (single, 3H, 2 OH, NH), $\delta$ 4.15 (multiple, 1H, NH-CH), $\delta$ 3.60 (multiple, 2H, $CH_2OH$). Analysis: (calc.) ($C_{11}H_{12}Cl_2N_2O_4$) C 43.00, H 3.94, N 9.12, Cl 23.10; (found) C 42.97, H 3.98, N 9.16, Cl 23.01.

**D(−) *threo*-2-Dichloroacetamido-1-*p*-(*N*-hydroxyacetamido)phenyl-1,3-propanediol (IV).**

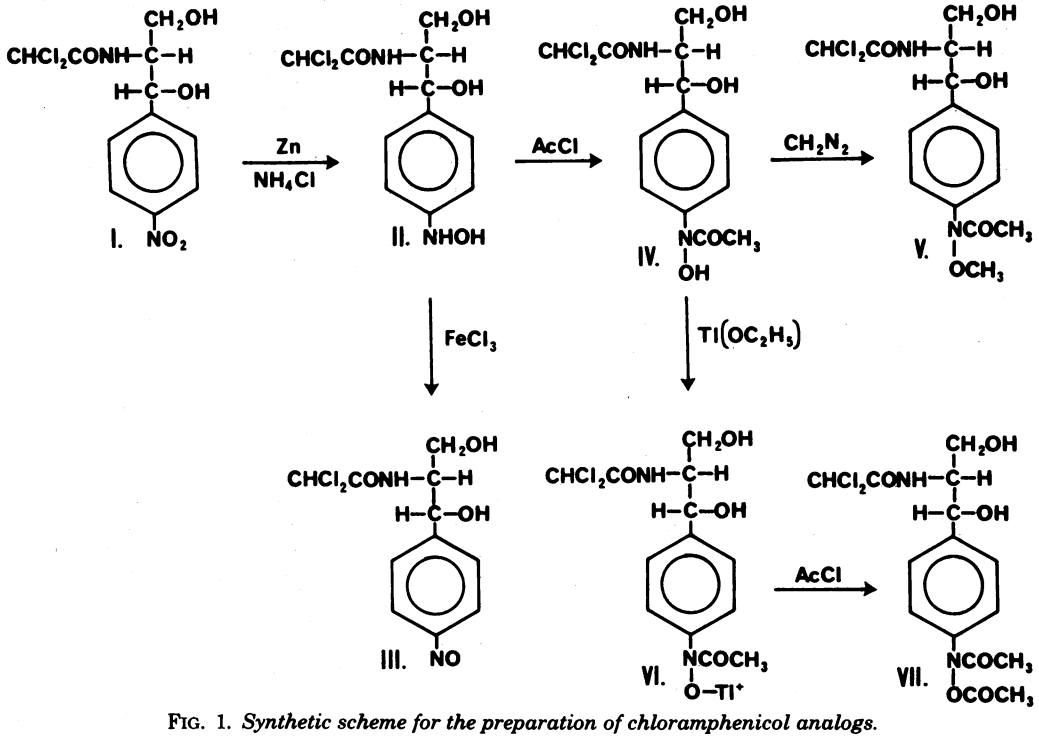


FIG. 1. *Synthetic scheme for the preparation of chloramphenicol analogs.*

The unpurified hydroxylamine (II, 2.0 g, 0.007 mol theoretical) was dissolved in 25 ml of $Et_2O$ and placed in a 100-ml round-bottom flask with $NaHCO_3$ (0.95 g, 0.011 mol) in 4 ml of water. The mixture was cooled to −5°C by means of an ice-salt bath and stirred vigorously while acetyl chloride (0.79 g, 0.01 mol) in 10 ml of anhydrous $Et_2O$ was added dropwise in the course of 10 min (23). The pale yellow suspension was stirred for an additional 10 min, and the $Et_2O$ layer was removed. The aqueous layer and accompanying oil were extracted three times with 25 ml of $Et_2O$. The $Et_2O$ fractions were combined, dried ($Na_2SO_4$), and evaporated to give a residue, which was dissolved in 10 ml of MeOH and 30 ml of $CHCl_3$ and then chromatographed on a silica gel (90 g) column (2 by 80 cm) with a gradient of 25 to 50% MeOH/$CHCl_3$. The fractions consisting of material that gave a single spot on TLC (silica gel with 10% MeOH/$CHCl_3$) with an $R_f$ of 0.2 and also gave a violet color with $FeCl_3$ spray reagent were combined and evaporated to give a hygroscopic semisolid. Further purification of this material was achieved by the use of the ferric-SP-Sephadex procedure (5) to give a solid material, which upon recrystallization ($Et_2O$/hexane) gave a white crystalline powder (1.4 g, 40%). mp 133 to 134.5°C; $UV_{max}^{EtOH}$ 259 nm (log $\epsilon$ 4.1); IR (KBr) 3,300 $cm^{-1}$ (OH, NOH), 1,675 $cm^{-1}$ (amide C=O); 1,640$cm^{-1}$ (hydroxamate C=O); NMR (220 MHz in $CD_3OD$) $\delta$ 7.46 (sym. multiple, 4H, $C_6H_4$), $\delta$ 6.27 (single, 1H, $CHCl_2$), $\delta$ 4.98 (double, 1H, $C_6H_4$-CH), $\delta$ 4.89 (single, 4H, 3 OH, 1 NH), $\delta$ 4.07 (AMX$_2$ pattern, 1H, NH-CH), $\delta$ 3.60 (AB X pattern, 2H, $CH_2OH$), $\delta$ 2.25 (broad single, 3H, $COCH_3$); mass spectrum (70 eV electron impact) mass measurement of $[M-18]^+$ at $m/e$ 332 gave 332.03378, consistent with the formula $C_{13}H_{14}N_2O_4Cl_2$ (theoretical 332.03306). Analysis: (calc.) ($C_{13}H_{16}Cl_2N_2O_5$) C 44.44, H 4.59, N 7.98, Cl 20.20; (found) C 44.17, H 4.59, N 7.78, Cl 19.96.

**D(−)-*threo*-2-Dichloroacetamido-1-*p*-(*N*-methoxyacetamido)phenyl-1,3-propanediol (V).** The purified hydroxamic acid (IV, 1.0 g, 0.003 mol) was dissolved in 5 ml of absolute EtOH and added to approximately 1.4 g of nondistilled $CH_2N_2$ in $Et_2O$ (1). After standing at 25°C for 2 h, excess $CH_2N_2$ was destroyed by the addition of acetic acid. The solvent was removed in vacuo to give a viscous oil, which was chromatographed on 60 g of silica gel with 30% $CH_3CN/CHCl_3$ to give a solid that recrystallized from EtOAc/$Et_2O$ to give V (0.82 g, 74%). mp 117 to 117.5°C; IR (KBr) 3,600 $cm^{-1}$ (OH), 3,240 $cm^{-1}$ (NH), 1,670 $cm^{-1}$ (2 C=O); NMR ($CD_3OD$) $\delta$ 7.48 (single, 4H, $C_6H_4$), $\delta$ 6.28 (single, 1H, $CHCl_2$), $\delta$ 5.09 (multiple, 1H, $C_6H_4$-CH), $\delta$ 4.70 (br. single, 3H, 2 OH, 1 NH), $\delta$ 4.08 (multiple, 1H, NH-CH), $\delta$ 3.68 (single, 3H, $OCH_3$), $\delta$ 3.60 (multiple, 2H, $CH_2OH$), $\delta$ 2.20 (single, 3H, $COCH_3$). Analysis: (calc.) ($C_{14}H_{18}N_2O_5Cl_2$) C 46.02, H 4.97, N 7.67, Cl 19.42; (found) C 46.03, H 5.03, N 7.61, Cl 19.40.

**D(−)-*threo*-2-Dichloroacetamido-1-*p*-(*N*-acetoxyacetamido)phenyl-1,3-propanediol (VII).** The purified hydroxamic acid (IV, 0.35 g, 0.001 mol) was dissolved in 5 ml of absolute EtOH; then 5 ml of benzene was added, and the solution was stirred while thallous ethoxide (250 mg, 0.001 mol) in 3 ml of benzene was added. The reaction was stirred for an additional 5 min and then filtered. The residue was washed with benzene/$Et_2O$ (1:1) to give 530 mg (96%) of the pale yellow thallous salt (mp 128 to 130°C, decomposed). The thallous salt was suspended in a 50-ml round-bottom flask with 20 ml of anhydrous $Et_2O$. The suspension was cooled and stirred in an ice-salt bath while acetyl chloride (78 mg, 0.001 mol) in 5 ml of anhydrous $Et_2O$ was added dropwise in the course of 5 min. The reaction mixture was stirred for an additional 15 min, warmed to room temperature, and filtered. The filtrate was evaporated in vacuo to give an oil, which was purified by chromatography on silica gel employing 5% MeOH/$CHCl_3$ to give 90 mg (23%) of a hygroscopic solid. TLC analysis indicated one major component ($R_f$ 0.61; 10% MeOH/$CHCl_3$ on silica gel), which slowly gave a violet color with $FeCl_3$ spray reagent upon standing. IR (film) 1,810 $cm^{-1}$ (N-O-$COCH_3$); NMR ($CD_3OD$) $\delta$ 7.49 (single, 4H, $C_6H_4$), $\delta$ 6.26 (single, 1H, $CHCl_2$), $\delta$ 5.12 (double, 1H, $C_6H_4$-CH), $\delta$ 4.83 (single, 5H, 20H, 1 NH, $H_2O$), $\delta$ 4.10 (multiple, 1H, NH-CH), $\delta$ 3.60 (multiple, 2H, $CH_2OH$), $\delta$ 2.19 (single, 3H, $COCH_3$), $\delta$ 2.05 (single, 3H, $COCH_3$). Although an accurate elemental analysis could not be obtained, the identity of the material was confirmed by using mild base hydrolysis in 2% $NaHCO_3$ at 50°C for 1 h to give the hydroxamic acid, IV.

**Determination of minimum inhibitory concentrations.** Compounds I through V and VII were dissolved in water just before testing to give concentrations of 1.0 mg/ml. Serial dilutions of each compound were made by first adding 1.0 ml of the test solution to 1.0 ml of sterile nutrient broth (Difco). This 2.0-ml solution was then added to a tube containing the test organism in 2.0 ml of nutrient broth to give the maximum test concentration of 250 $\mu$g/ml. The dilutions were then made by transferring 2.0 ml from this first tube through a series of tubes containing 2.0 ml of inoculated nutrient media. A total of 10 concentrations were tested for each compound for inhibition of *Pseudomonas aeruginosa* (ATCC 15442, *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536), and *Bacillus subtilis* (ATCC 6633). In addition, the hydroxamic acid (IV) was investigated at 10-fold-higher concentrations. The tubes were examined for growth after incubation at 37°C for 48 h. The minimum concentration of each compound that completely inhibited bacterial growth is recorded in Table 1.

**Stability of chloramphenicol analogs under incubation conditions.** Each analog was dissolved in 10 ml of sterile nutrient broth to give a concentration

TABLE 1. *Minimum inhibitory concentrations (MIC) for chloramphenicol and five analogs against four bacterial strains*

| Compound | MIC (μg/ml) | | | |
|---|---|---|---|---|
| | *S. aureus* | *P. aeruginosa* | *E. coli* | *B. subtilis* |
| I | 0.007 | 0.003 | 0.001 | 0.001 |
| II | 0.062 | 0.062 | 0.125 | 0.062 |
| III | 0.062 | 0.062 | 0.062 | 0.062 |
| IV | >2.5 | >2.5 | >2.5 | 2.5 |
| V | 0.250 | 0.062 | >0.250 | 0.062 |
| VII | >0.250 | >0.250 | >0.250 | >0.250 |

of 0.25 mg/ml. After incubation at 37°C for 24 h, the solutions were extracted twice with EtOAc. The combined EtOAc extracts were dried ($Na_2SO_4$) and evaporated. TLC analysis (silica gel with 10% MeOH/$CHCl_3$) of each residue was conducted to determine the presence of the original test compound, and the nature and degree of any degradation products, particularly chloramphenicol. All expected decomposition products had been previously shown to be extractable into EtOAc from aqueous solutions.

## RESULTS

The nitroso (III), hydroxylamine (II), hydroxamic acid (IV), *O*-methyl hydroxamate ester (V), and *O*-acetyl hydroxamate (VII) analogs of chloramphenicol were prepared and characterized by spectral methods. A selective method for the acylation of the hydroxamic acid hydroxyl group in the presence of alcohol functional groups was developed. This method consisted in the conversion of the hydroxamic acid group to its thallous salt, which selectively reacted with acetyl chloride. The hydroxylamine (II) and hydroxamic acid (IV) analogs were extremely water soluble, which made their purification rather difficult; in fact, the hydroxylamine (II) has not yet been obtained in sufficient purity to allow for an accurate elemental analysis. The material employed for the antimicrobial testing of II was, however, devoid of any detectable chloramphenicol impurity and did give a single spot on TLC analysis. The only other analog that could not be obtained in analytical purity was the *O*-acetyl hydroxamate analog (VII). This compound was hygroscopic in addition to being rather unstable. The instability of VII prevented extensive chromatographic purification other than to obtain a product that gave a single spot by TLC analysis. NMR analysis conservatively indicated that II and VII were of 95% purity, while the remaining three analogs had purities in excess of 99%.

The nitroso analog (III) was unusual in that it gave bright blue crystals, indicating that it exists in the monomeric form in the solid state (8). This is an unusual feature for aromatic nitroso compounds, which usually display a blue or green color only in solution as the result of an $n \rightarrow \Pi^*$ transition of the monomeric nitroso group. However, an amorphous form of III was also observed which was nearly colorless, and thus probably consisted of the dimeric nitroso form (8).

Stability testing of each analog under conditions employed for antibiotic testing demonstrated that none of the analogs was converted to chloramphenicol, an event which would have given erroneous results in the biological testing. With the exception of the *O*-acetyl hydroxamate analog (VII), all analogs underwent only minor decomposition to unidentified products in the 24-h period as estimated by TLC analysis. We were particularly surprised that the hydroxylamine (II) was not converted in appreciable amounts to chloramphenicol, in view of other reports on the instability of aromatic hydroxylamines (12, 21). Analog VII underwent extensive hydrolysis to give the free hydroxamic acid (IV). Thus, the data recorded in Table 1 for this unstable compound may not reflect the true potential antibiotic properties of this analog.

The results of the antibiotic testing against four bacteria are recorded in Table 1. Chloramphenicol was employed as a positive control and to provide a basis for comparison of the activities of the various analogs. The hydroxamic acid (IV) and its *O*-acetyl derivative (VII) lacked any activity at the maximum concentrations employed. The conversion of VII to IV would explain the lack of antibiotic activity of the former. The remaining analogs were an order of magnitude less active than chloramphenicol. Most disappointing was the failure to observe strong antibiotic activity on the part of the nitroso analog (III).

## DISCUSSION

Microbial reduction of aromatic nitro compounds to aromatic amines is known to proceed with the intermediate formation of the nitroso and hydroxylamine compounds, which at times can be detected in appreciable concentrations (19). Thus, the hydroxylamine (II) and nitroso (III) analogs of chloramphenicol are likely, although probably fleeting, metabolites of this antibiotic. It is known that the aniline analog of chloramphenicol is an important metabolite, readily detectable in urine (7). In view of recent reports on the metabolism of aromatic nitro compounds in vivo, it appears that bacterial reduction of the nitro group of chloramphenicol to give the amine metabolite is probably much more significant than nitro group reduction by mammalian tissues (22, 27). In fact, there is reason to believe that nitroreductase is not a significant enzymatic process in aerobic mammalian tissues, since mammalian nitroreductase is strongly inhibited by oxygen (18, 24). On the other hand, the oxidation of the known amine metabolite of chloramphenicol, either by tissue microsomal oxidases (11, 20) or by the gastrointestinal microflora, might also produce the hydroxylamine (II) and nitroso (III) compounds as transient metabolites. It is well known that the induction of methemoglobinemia by aromatic amine and nitro compounds is the result of a biological oxidation-reduction interconversion of the corresponding nitroso and hydroxylamine oxidation states (13, 19).

Literature precedence is so overwhelming that we felt that the hydroxylamine (II) and nitroso (III) analogs could not be overlooked as important mediators of part or all of the biological actions of chloramphenicol. Furthermore, there is sufficient precedence to suspect that the hydroxamic acid analog (IV) of chloramphenicol might also be a metabolite, since the metabolic conversion of hydroxylamines (26) and nitroso (3, 4) compounds to hydroxamic acids is known to occur. The fact that such compounds are not known urinary metabolites of chloramphenicol is by no means an argument against their formation in vivo.

Our antibiotic testing results revealed that none of these potential metabolites possessed any appreciable activity when compared with chloramphenicol. The hydroxamic acid analog (IV) was devoid of antibiotic activity, even at concentrations as high as 2.5 μg/ml. In this case we suspect that the lack of any activity is possibly a result of the hydrophilic nature of the hydroxamic acid, which could prevent its penetration into the microbial cell. Conversion of the hydroxamic acid (IV) to its methyl ester (V) greatly decreases this hydrophilicity and results in the reappearence of modest antibiotic activity in analog V. This proposal is consistent with the known fact that activity in the chloramphenicol series is dependent upon the presence of a hydrophobic functional group at the 4-position of the aromatic ring (6, 10).

Most notable was our observation that the nitroso analog (III) is not nearly as potent an antibiotic as is chloramphenicol, yet the solubility properties of the nitroso analog (III) are identical to those of chloramphenicol. We had considered the possibility that the nitroso analog (III) might actually be more potent than chloramphenicol by virtue of the chemical reactivity of the nitroso group (14, 16) compared with the relative inertness of the nitro group of chloramphenicol. In view of our results, we conclude that chemical reactivity at the nitro position is of no importance in the expression of antibiotic activity in the chloramphenicol series. Such a conclusion is substantiated by a consideration of the structure of thiamphenicol, which is a potent antibiotic. It remains to be demonstrated whether such chemical reactivity at the nitro position is important to the occurrence of toxic side effects in the chloramphenicol series. In this case, the relatively nontoxic properties of thiamphenicol definitely suggests this possibility.

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## LITERATURE CITED

1. **Arndt, F.** 1943. Diazomethane. Org. Syn. Coll. **2**:165–167.
2. **Bonanomi, L., D. Della Bella, and A. Gazzaniga.** 1975. Newer developments in chloramphenicol metabolism. Pharmacol. Res. Commun. **7**:437–441.
3. **Corbett, M. D., J. E. Cahoy, and B. R. Chipko.** 1975. Conversion of nitrosobenzene to N-phenylacetohydroxamic acid by yeast pyruvate decarboxylase. J. Natl. Cancer Inst. **55**:1247–1248.
4. **Corbett, M. D., and B. R. Chipko.** 1977. N-phenylglycolhydroxamate production by the action of transketolase on nitrosobenzene. Biochem. J. **165**:263–267.
5. **Corbett, M. D., and B. R. Chipko.** 1978. The purification of hydroxamic acids by the use of ferric SP-Sephadex. J. Chromatogr., in press.
6. **Freeman, K. B.** 1977. Action of *N*-trifluoroacetyl analogue of D-chloramphenicol. Antimicrob. Agents Chemother. **11**:563–565.
7. **Glazko, A. J.** 1967. Identification of chloramphenicol metabolites and some factors affecting metabolic disposition, p. 655–665. Antimicrob. Agents Chemother. 1966.
8. **Gowenlock, B. G., and W. Lüttke.** 1958. Structure and properties of C-nitroso compounds. Q. Rev. **12**:321–340.
9. **Hahn, F. E., J. E. Hayes, C. L. Wisseman, H. E. Hopps, and J. E. Smadel.** 1956. Mode of action of chloramphenicol. VI. Relation between structure and activity in the chloramphenicol series. Antibiot. Chemother. **6**:531–543.
10. **Hansch, C., K. Nakamoto, M. Gorin, P. Denisevich, E. R. Garrett, S. M. Heman-Ackah, and C. H. Won.** 1973. Structure-activity relationship of chloramphenicols. J. Med. Chem. **16**:917–922.
11. **Hlavica, P., and M. Kehl.** 1976. Comparative studies on the N-oxidation of aniline and N, N-dimethylaniline by rabbit liver microsomes. Xenobiotica **6**:679–689.
12. **Kadlubar, F. E., J. A. Miller, and E. C. Miller.** 1976. Microsomal N-oxidation of the hepatocarcinogen N-methyl-4-aminoazobenzene and the reactivity of N-hydroxy-N-methyl-4-amino azobenzene. Can. Res. **36**: 1196–1206.
13. **Kiese, M., and K. Taeger.** 1976. The fate of phenylhydroxylamine in human red cells. Arch. Pharmacol. **292**:59–66.
14. **King, C. M., N. R. Traub, R. A. Cardona, and R. B. Howard.** 1976. Comparative adduct formation of 4-aminobiphenyl and 2-aminofluorene derivatives with macromolecules of isolated liver parenchymal cells. Can. Res. **36**:2374–2381.
15. **Krishna, G.** 1974. Covalent binding of drugs to tissue macromolecules as a biochemical mechanism of drug toxicities with special emphasis on chloramphenicol and thiamphenicol. Postgrad. Med. J. **50**(Suppl. 5):73–77.
16. **Lotlikar, P. D., E. C. Miller, J. A. Miller, and A. Margreth.** 1965. The enzymatic reduction of the N-hydroxy derivatives of 2-acetylaminofluorene and related carcinogens by tissue preparations. Can. Res. **25**:1743–1752.
17. **Man-yan, D. R., G. K. Arimura, and A. A. Yunis.** 1975. Comparative metabolic effects of chloramphenicol analogues. Mol. Pharmacol. **11**:520–527.
18. **Mason, R. P., and J. L. Holtzman.** 1975. The mechanism of microsomal and mitochondrial nitroreductase. Electron spin resonance evidence for nitro aromatic free radical intermediates. Biochemistry **14**:1626–1632.
19. **Michard, M.** 1972. Bioreduction of organic nitrogen, p. 157–169. *In* J. W. Bridges, J. W. Gorrod, and D. V. Parke (ed.), Biological oxidation of nitrogen in organic

molecules. Halsted Press, New York.
20. **Prough, R. A., and D. M. Ziegler.** 1977. The relative participation of liver microsomal amine oxidase and cytochrome P-450 in N-demethylation reactions. Arch. Biochem. Biophys. **180**:363–373.
21. **Radomski, J. L., A. A. Rey, and E. Brill.** 1973. Evidence for a glucuronic acid conjugate of N-hydroxy-4-aminobiphenyl in the urine of dogs given 4-aminobiphenyl. Can. Res. **33**:1284–1289.
22. **Reddy, B. G., L. R. Pohl, and G. Krishna.** 1976. The requirement of the gut flora in nitrobenzene-induced methemoglobinemia in rats. Biochem. Pharmacol. **25**:1119–1122.
23. **Smissman, E. E., and M. D. Corbett.** 1972. A facile method for N-acylation of ring activated phenylhydroxylamines. J. Org. Chem. **37**:1847–1849.
24. **Wardman, P., and E. D. Clarke.** 1976. Oxygen inhibition of nitroreductase: electron transfer from nitro radical-anions to oxygen. Biochem. Biophys. Res. Commun. **69**:942–949.
25. **Weisburger, J. H., Y. Shirasu, P. H. Grantham, and E. K. Weisburger.** 1967. Chloramphenicol, protein synthesis, and the metabolism of the carcinogen N-2-fluorenyldiacetamide in rats. J. Biol. Chem. **242**:372–378.
26. **Weisburger, J. H., and E. K. Weisburger.** 1973. Biochemical formation and pharmalogical, toxicological and pathological properties of hydroxylamines and hydroxamic acids. Pharmacol. Rev. **25**:1–66.
27. **Wheeler, L. A., F. B. Soderberg, and P. Goldman.** 1975. The relationship between nitro group reduction and the intestinal microflora. J. Pharmacol. Exp. Ther. **194**:135–144.
28. **Yunis, A. A.** 1974. Chloramphenicol toxicity, p. 107–126. *In* R. H. Girdwood (ed.), Blood disorders due to drugs and other agents. Excerpta Medica, Amsterdam.
29. **Yunis A. A., and G. R. Bloomberg.** 1964. Chloramphenicol toxicity: clinical features and pathogenesis. Prog. Hematol. **4**:138–159.