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# Light Sensitive Chloramphenicol Analogues

PETER E. NIELSEN,[a] VAGN LEICK [b] and OLE BUCHARDT [a]

[a] Chemical Laboratory II, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark and [b] Biochemical Institute B, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

Some chloramphenicol* analogues have been prepared in order to find reagents which could be used in a photoaffinity labelling study of the peptidyl transferase center of the *E. coli* ribosome. The compounds were tested for antibiotic activity *in vitro* in the "fragment reaction" assay, and for their ability to generate very reactive species upon irradiation.

The bacteriostatic action of many antibiotics, *inter alia*, chloramphenicol, is believed to be due to competition with the aminoacylated end of aminoacyl-t-RNA at the A-site on 70 S type ribosomes.[1]

Previously attempts have been made to identify the particular macromolecule(s) involved in the peptidyl transferase center by affinity labelling with derivatives of chloramphenicol (I).[2,3] In these studies, monobromamphenicol (VII) [2] and monoiodamphenicol (IX) [3] were employed. However, in the one report,[2] the antibiotic was irreversibly attached to the L 16 protein of the ribosome, whereas in the other case,[3] the antibiotic was attached to proteins L 2 and L 27 of the ribosome.

In an attempt to clarify this ambiguity we decided to prepare a number of chloramphenicol analogues in order to undertake a photoaffinity labelling study of the peptidyl transferase center. In this paper the preparation of chloramphenicol analogues as well as some of their antibiotic properties is reported.

The principle of photoaffinity labelling consist in letting a traceable, photoactive and biologically active reagent interact with its receptor site in a biological molecule. Upon irradiation with light of a suitable wavelength the reagent should be irreversibly and specifically attached at the active site, and by fractionating and analyzing the products from this, information about the location of the active site can be obtained.[4]

Thus we decided to prepare a series of chloramphenicol analogues, which besides showing biological activity also were photochemically active. In particular we were interested in designing compounds which could be expected to give nitrenes or carbenes upon photolysis.

According to the literature, the biological activity of chloramphenicol analogues is depending on: (*a*) the correct stereochemistry; (*b*) the presence of the two hydroxy groups; (*c*) the acetamido group, substituted with small electronegative substituents; (*d*) an electron withdrawing group in the 4-position of the phenyl group.

On the basis of this, we decided to modify the 4-substituent in the phenyl group or the acyl group in the aliphatic side chain.

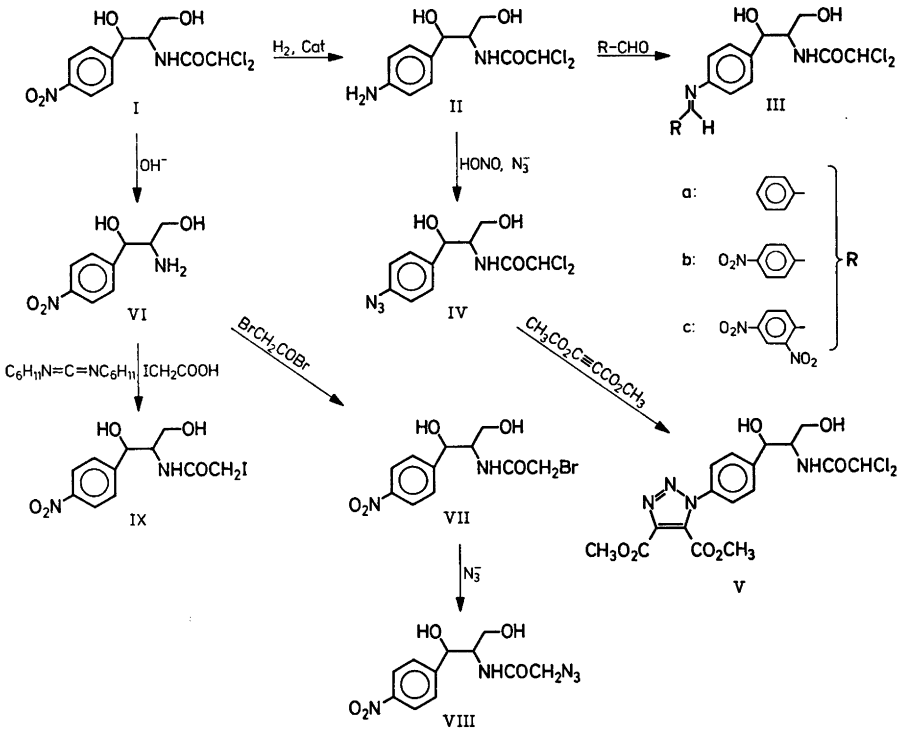
## RESULTS AND DISCUSSION

By conventional, and in most cases well known procedures, the compounds listed in Scheme 1 were prepared. The synthetic pathways are also outlined in Scheme 1.

Compounds II,[6] IIIa,[7] IIIb,[6] VI,[8] VII,[9] and VIII [9] were previously known. Compounds IIIc, IV, and V which were not previously known, were identified on the basis of their elemental analysis and their spectroscopic characteristics.

The photochemical activity of the chloramphenicol analogues was examined, and IIIa,

* (1*R*:2*R*)-2-(Dichloroacetamido)-1-(4-nitrophenyl)-1,3-propandiol.




*Scheme 1.*

IIIb, IIIc, IV, and V were all found to be relatively active (Table 1). In no instances were the photoproducts isolated and characterized. However, it was found for the aromatic azide (IV), that an apparently clean light-induced reaction took place in methanol in $\sim 10^{-4}$ M concentration (Fig. 1), whereas irradiation of more concentrated solutions led to a complex mixture of products.

*Table 1.* Photoactivity of compounds.

| Compound | Quantum yield [a] |
|---|---|
| IIIa | 0.2 |
| IIIb | < 0.1 |
| IIIc | < 0.1 |
| IV | 1 |
| V | 0.25 |
| VIII | < 0.02 |

[a] Relative to compound IV, estimated from rate of photolysis.

Interestingly, the Schiff bases (III) all exhibited good photoreactivity.

The aliphatic azide (VIII) showed a surprisingly small photoreactivity, as compared to

*Table 2.* Antibiotic activity of compounds.

| Compound | % activity [a] | % antibacterial activity |
|---|---|---|
| Chloramphenicol (I) | 100 | 100 |
| II | 2 | 0 [5] |
| IIIa | 2 | 1 [12] |
| IIIb | 2 | 45 [12] |
| IIIc | 2 | |
| IV | 10 | |
| V | 5 | |
| VI | < 0.5 | 1.8 [5] |
| VII | ~ 20 | 15 [5] |
| VIII | 20 | |
| IX | 10 | |

[a] Relative to chloramphenicol in fragment reaction assay.

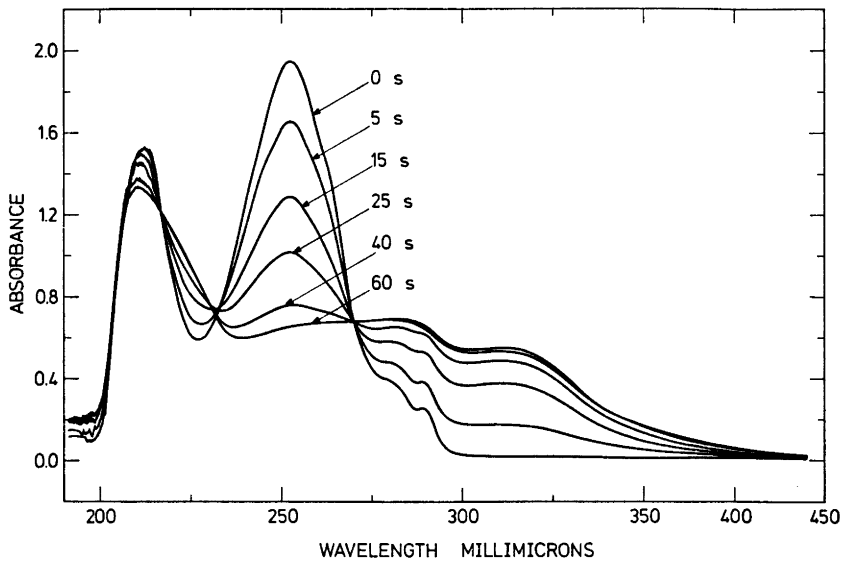



*Fig. 1.* Photolysis of compound IV.

other less complicated aliphatic azides.[10] This must probably be due to intramolecular energy transfer from the azide chromophore to the aromatic nucleus.

The inhibition of the peptidyl transferase activity of 70 S *E. coli* ribosomes in the fragment reaction [11,13] was determined for the chloramphenicol analogues (Table 2).

From Table 2 it is seen that the inhibition by compounds II, IIIa, VI, and VII are in good agreement with previous antibacterial determinations. However, compound IIIb shows a considerably smaller activity in our assay than that previously recorded.[12]

This difference may be due to the different assay systems.

## CONCLUSION

Compound IV and to a smaller extent compound V appeared usable for a photoaffinity labelling study, whereas the other chloramphenicol analogues seem to be unsuitable. Unfortunately the photoreactions of compounds IV and V in methanol solution, in the absence of biological material, required light of such short wavelength that the ribosomes also absorb strongly. Thus it might be expected that eventual excited photoaffinity labels would transform the excitation to the ribosome. However, if the photoreaction was sufficiently fast they would react before energy transfer took place. The photoaffinity labelling experiments will be published in a future paper.

## EXPERIMENTAL

*Photochemical activity.* In order to test the compounds for photochemically activity they were irradiated with UV-light (B & L mercury SP 200 light source) in quartz-cuvettes (MeOH; 0.03 mg/ml) and the reaction followed by UV-spectroscopy.

*Test for antibiotic activity.* The antibiotic activity of the compounds was determined as their ability to inhibit the peptidyl-transferase activity of 70 S ribosomes from *E. coli*, assayed in the "fragment reaction" as described by Monro.[13] 70 S ribosomes (*E. coli* strain MRE 600) were prepared according to Anderson *et al.*[11] and Ac-$^3$H-leu-pentanucleotide according to Monro.[13] The reaction volume was 200 $\mu$l, containing 50 % MeOH, v/v, 5 $\mu$mol of Tris.HCl buffer, (pH 7.5), 2 $\mu$mol of $Mg^{2+}$, 0.04 $\mu$mol of ethanethiol, 0.1 $\mu$mol of puromycin. HCl, 0.2 mg ribosomes, 2000 – 3000 cpm of the "fragment" and $10^{-5} - 10^{-2}$ $\mu$mol of the compound to be tested.

Melting points were determined on a Reichardt melting point microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer 337 infrared spectrophotometer and UV spectra on a Unicam SP 1800 UV

spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

*(1R:2R)-2-(Dichloroacetamido)-1-(4-aminophenyl)-1,3-propandiol. hydrochloride (II).* Chloramphenicol (I) (4 g) was dissolved in methanol (240 ml), containing conc. HCl (3.6 ml), and 10 % Pd/C catalyst (300 mg). The mixture was kept at 0 °C under magnetic stirring and hydrogenated for 2½ h, at which time the absorbtion rate of $H_2$ had decreased considerably (850 ml $H_2$ was absorbed). The catalyst was filtered off, the solvent evaporated, and the crystalline residue washed with dry ethanol (2 ml) and diethyl ether (20 ml) to yield 3.8 g (95 %) hydrochloride. M.p. 130 °C decomp. $[\alpha]_D^{20}$ +4.6° (MeOH, *c* 2.05). (Found: C 39.65; H 4.59; N 8.36; Cl 31.28. Calc. for $C_{11}H_{15}O_3N_3Cl_3$ C 40.06; H 4.55; N 8.49; Cl 32.32). IR (KBr): $cm^{-1}$ 2600 (m), 1660 (s).

*(1R:2R)-2-(Dichloroacetamido)-1-(4-benzylideniminophenyl)-1,3-propandiol (IIIa).* Compound II (500 mg) was dissolved in $H_2O$ (2 ml) and neutralized with an excess of $KHCO_3$. Ethanol (6 ml) was added, followed by benzaldehyde (0.6 ml). After 30 min at room temperature, precipitation of the Shiff base began, and it was completed with addition of water at 0 °C. The crystals were filtered off, and dried in vacuum. Yield: 300 mg (51 %). M.p. 162–163 °C (lit.[7] 153–154 °C). $[\alpha]_D^{20}$ +7.5° (MeOH, *c* 0.82), −45.6° (acetone, *c* 0.49). (Found: C 56.48; H 4.81; N 7.23; Cl 18.71. Calc. for $C_{18}H_{18}O_3N_2Cl_2$: C 56.69; H 4.72; N 7.34; Cl 18.63). UV (MeOH): $\lambda_{max}$ nm (log $\varepsilon$) 335 (2.14), 272 (2.37). IR (KBr): $cm^{-1}$ 1680 (s).

*(1R:2R)-2-(Dichloroacetamido)-1-[4-(4-nitrobenzylidenimino)phenyl]-1,3-propandiol (IIIb).* Compound II (400 mg) was dissolved in $H_2O$ (2 ml) and neutralized with an excess of $KHCO_3$. To this mixture was added ethanol (10 ml) containing *p*-nitrobenzaldehyde (200 mg). The reaction vessel was protected from light and incubated for 72 h at room temperature. Thereafter the Shiff base was precipitated with $H_2O$ and filtered off to yield 400 mg (75 %) of the title compound. Recrystallization from 1,2-dichloroethane. M.p. 166–168 °C (lit.[6] 164–166 °C). $[\alpha]_D^{20}$ +8.1° (MeOH, *c* 0.46). (Found: C 50.69; H 4.14; N 9.61. Calc. for $C_{18}H_{17}O_5N_3Cl_2$: C 50.70; H 3.99; N 9.86). UV (MeOH): $\lambda_{max}$ nm (log $\varepsilon$) 348 (2.30), 262 (2.48). IR (KBr): $cm^{-1}$ 1680 (s), 1510 (s), 1350 (s).

*(1R:2R)-2-(Dichloroacetamido)-1-[4-(2,4-dinitrobenzylidenimino)phenyl]-1,3-propandiol (IIIc).* This compound was made by condensing compound II with 2,4-dinitrobenzaldehyde analogous to the procedure from compound IIIb. Yield: 350 mg (60 %). Recrystallization from 50 % ethanol. M.p. 144–146 °C. $[\alpha]_D^{20}$ −26.5° (acetone, *c* 0.76). (Found: C 45.80; H 3.87; N 12.13. Calc. for $C_{18}H_{16}O_7N_4Cl_2$: C 45.6; H 3.60; N 11.80). UV (MeOH): $\lambda_{max}$ nm (log $\varepsilon$) 360 (2.16), 244 (2.72). IR (KBr): $cm^{-1}$ 1675(s), 1520(s), 1340(s).

*(1R:2R)-2-(Dichloroacetamido)-1-(4-azidophenyl)-1,3-propandiol (IV).* Compound II (1 g) was dissolved in ice cold 2 N $H_2SO_4$ (12 ml). The solution was stirred, and $NaNO_2$ (400 mg) was added in portions during 60 min, followed by $NaN_3$ (500 mg) during 45 min. Simultaneously with the $NaN_3$ addition, ethyl acetate (10 ml) was poured into the mixture to make a two phase system. The phases were then separated, and another extraction with ethyl acetate (10 ml) was undertaken. The combined ethyl acetate fractions were dried over $MgSO_4$ and the solvent evaporated to yield 600 mg (66 %) of the title compound. Recrystallization was made from 1,2-dichloroethane. M.p. 89–92 °C. $[\alpha]_D^{20}$ +5.8° (MeOH, *c* 1.32), −34.6° (acetone, *c* 0.52). (Found: C 41.07; H 3.79; N 17.31; Cl 22.25. Calc. for $C_{11}H_{12}O_3N_4Cl_2$: C 41.37; H 3.76; N 17.55; Cl 22.25). UV (MeOH): $\lambda_{max}$ nm (log $\varepsilon$) 252 (2.49). IR (KBr): $cm^{-1}$ 2110 (s), 1680 (s).

*(1R:2R)-2-(Dichloroacetamido)-1-[4-(4,5-dimethoxycarbonyl-1,2,3-triazolyl)phenyl]-1,3-propandiol (V).* Compound IV (200 mg) was dissolved in dry ethanol (6 ml) and refluxed with dimethyl acetylenedicarboxylate (200 mg) for 3 h. The ethanol was removed under reduced pressure, and the residue washed with diethyl ether to yield 170 mg (60 %) of the title compound. Recrystallization from 1,2-dichlorethane. M.p. 174–176 °C. $[\alpha]_D^{20}$ −23.8° (acetone, *c* 0.42). (Found: C 43.83; H 4.01; N 12.13. Calc. for $C_{17}H_{16}O_7N_4Cl_2$: C 44.25; H 3.90; N 12.14). UV (MeOH): $\lambda_{max}$ nm (log $\varepsilon$) 242 (2.47). IR (KBr): $cm^{-1}$ 1720 (s), 1670 (s).

*(1R:2R)-2-(Azidoacetamido)-1-(4-nitrophenyl)-1,3-propandiol (VIII).* This compound was made according to Rebstock [9] starting from base hydrolyzed chloramphenicol yielding (1*R*:2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propandiol (VI). M.p. 102–103 °C (lit.[9] 120–121 °C for the DL-form). $[\alpha]_D^{20}$ −15.0° (acetone, *c* 0.99). UV (MeOH): $\lambda_{max}$ nm (log $\varepsilon$) 272 (2.49). IR (KBr): $cm^{-1}$ 2100 (s), 1660 (s), 1500 (s) 1340 (s).

*Hydrolysis.* A suspension of chloramphenicol (7 g) in 0.25 N NaOH (170 ml) was stirred for 2 h at room temperature, chilled to 0 °C and the precipitate filtered off. One recrystallization from $H_2O$ yielded the pure chloramphenicol base (VI) (3.5 g, 75 %). M.p. 162–163 °C (lit.[8] 161–162 °C).

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