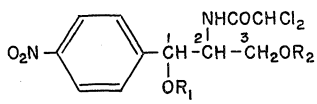
MICROBIAL TRANSFORMATION OF ANTIBIOTICS
VI. ACYLATION OF CHLORAMPHENICOL BY *STREPTOMYCES COELICOLOR*

Sir:

Previous papers in this series have described the microbial transformation of clindamycin to clindamycin-3-phosphate[1], N-demethylclindamycin[2], clindamycin sulfoxide[2] or clindamycin ribonucleotides[3] by *Streptomyces* species. We have extended our observations on the ability of these organisms to transform antibiotics, to chloramphenicol (I, Fig. 1) which, like clindamycin, binds at the 50 S ribosomal subunit in bacteria and inhibits protein synthesis. The present communication describes the transformation of chloramphenicol by *Streptomyces coelicor* MÜLLER.

Addition of chloramphenicol (100~500 mcg/ml) to cultures of *S. coelicolor* grown for 24 hours in a complex medium resulted in transformation of the antibiotic to compounds (as shown by TLC) lacking *in vitro* antibacterial activity against test organisms. Approximately 60~80 % of the antibiotic was inactivated under the conditions used. Mild alkaline treatment of an extract of the "inactivated broth" resulted in re-generation of chloramphenicol, suggesting[4] acylation of the antibiotic by *S. coelicolor*.

Fig. 1



	R_1	R_2
Chloramphenicol (I)	-H	-H
Compound II	$-COCH_3$	H
Compound III	-H	$-COCH_3$
Compound IV	-H	$-COCH_2CH_3$
Compound Va	-H	$-COCH(CH_3)_2$
Compound Vb	-H	$-COCH_2CH(CH_3)_2$

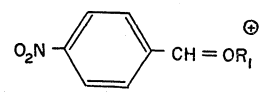
The chloramphenicol transformation products were isolated from the clear filtrate by solvent extraction followed by countercurrent distribution. The distribution was analyzed by U. V. and bioactivity determinations. Five U. V. peaks were detected, designated I to V; of these, only peak I was found to contain bioactive material. Material from the remaining peaks all afforded chloramphenicol following mild alkaline treatment[4].

Compound I (from peak I), was identified as chloramphenicol by direct comparison to an authenic sample.

Compounds II and III (crystalline from peaks II and III respectively) had identical molecular formulas (Table 1), differing from the formula of chloramphenicol by C_2H_3O. Both compounds showed ester carbonyl absorptions suggesting isomeric chloramphenicol acetate esters.

The N. M. R. spectra of chloramphenicol and compounds II and III (Table 2) showed a doublet of doublets of area 4 at δ 7.45~8.25 assigned to the aromatic hydrogens. Also the singlet at δ 6.45 ($-CH\langle^{Cl}_{Cl}$) and the complex absorption at *ca.* δ 4.0 (H at C-2) are common for all three materials. Two points distinguish the spectra of compound II from that of chloramphenicol. First a sharp singlet at δ 2.10 of area 3, not present in the spectrum of chloramphenicol, is assigned to the methyl group of the acetate esters; second the doublet at δ 5.1 assigned to the

Fig. 2



A: R_1=H

B: R_1=$COCH_3$

Table 1. Characterization data

Compound	Molecular formula	U. V.* [λ_{max}($\varepsilon\times10^{-3}$)]	Carbonyl area I. R. absorptions (cm^{-1})	Tentative structural assignment
I	$C_{11}H_{12}N_2O_5Cl_2$	273 (9.85)	1680; 1600; 1530	Chloramphenicol
II	$C_{13}H_{14}N_2O_6Cl_2$	267 (10.34)	1745; 1690; 1605; 1525	Chloramphenicol-acetate
III	$C_{13}H_{14}N_2O_6Cl_2$	270 (10.55)	1730; 1680; 1600; 1525	Chloramphenicol-acetate
IV	$C_{14}H_{16}N_2O_6Cl_2$	270 (10.21)	1730; 1680; 1600; 1525	Chloramphenicol-propionate
V	$C_{15}H_{18}N_2O_6Cl_2$	270 (9.80)	1730; 1680; 1600; 1525	Chloramphenicol-butyrate or isobutyrate

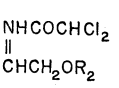
* U.V. spectra were obtained in 95 per cent ethanol.

Table 2. NMR* spectra of chloramphenicol and compounds II and III

Chemical shift (δ, ppm)			Type of absorption	Area	Assignment
Chloramphenicol	II	III			
7.45~8.25	7.45~8.25	7.45~8.25	Two doublets	4	Aromatic H's
6.45	6.45	6.45	Singlet	1	$-\underline{CH}Cl_2$
5.1	6.0	5.1	Doublet	1	H at C-1
ca. 4.0	*ca.* 4.0	*ca.* 4.0	Complex	1	H at C-2
ca. 3.5	*ca.* 3.5	*ca.* 4.2	Complex	2	2H at C-3
—	2.1	2.0	Singlet	3	$-OCO-\underline{CH_3}$

* N.m.r. spectra were observed with a Varian A-60 spectrometer on solutions (*ca.* 0.4 ml, *ca.* 0.25 M) of the compounds in D_6-dimethyl sulfoxide.

Fig. 3



C: R_2=H
D: R_2=$-COCH_3$
E: R_2=$-COCH_2CH_3$
F: R =$-COCH(CH_3)_2$
G: R_2=$-COCH_2CH(CH_3)_2$

hydrogen at C-1 has been shifted to δ 6.0 suggesting the presence of the acetate group at this carbon; that is, compound **II** is chloramphenicol-1-acetate (Fig. 1). Similarly, the spectrum of compound **III** shows a singlet of area 3 at δ 2.0 due to the methyl of the acetate ester group. In addition, the complex absorption at δ 3.5 due to the two hydrogens at C-3 of chloramphenicol has shifted to δ 4.2 indicating a chloramphenicol-3-acetate (Fig. 1) structure for this compound.

The mass spectra of compounds **II** and **III** showed M+1 peaks at 365, 367, 369 mass units. The isotopic cluster observed as well as the isotopic ratio of these ions differing by 2 mass units are characteristic of the 2 chlorine atoms known to be present in chloramphenicol.

Compound **II** has been assigned the chloramphenicol-1-acetate structure. This is confirmed by the ionic cluster peaks at 333 ($M-CH_2OH$) and at 170 (fragment C, Fig. 3) mass units. The peak at 195 assigned to fragment B (Fig. 2) plus one hydrogen further substantiates this conclusion. The mass spectral confirmation of the chloramphenicol-3-acetate structure in compound **III** is based on the ionic cluster fragments appearing at 291 ($M-CH_2OCOCH_3$) and 212 (fragment D, Fig. 3) mass units indicating the presence of the acetyl group at C-3. In addition, the peak at 152 (fragment A, Fig. 2) indicates that the secondary hydroxyl group is free.

Compound **IV**, is assigned the 3-propionate structure (Fig. 1) on the basis of analytical data and I. R. and U. V. spectra (Table 1). Furthermore, the N. M. R. spectrum of this material has a triplet at δ 1.05 of area 3 and a quadruplet at *ca.* δ 2.3 of area 2, in agreement with the propionate structure. In addition, the complex absorption of δ 3.5 in the spectrum of chloramphenicol, due to 2H at C-3, has shifted to δ 4.2 in the spectrum of **IV** locating the propionyl ester grouping at C-3. The mass spectra which showed ionic clusters at 379, 381, 383 (M+1), 291 ($M-CH_2OCOCH_2CH_3$) and 226 (fragment E, Fig. 3) mas units indicate that the propionyl group is attached to the primary hydroxyl group at C-3. This agrees with the presence of a peak at 152 mass units assigned to A (Fig. 2).

Preparation **V** was obtained from the last countercurrent distribution peak as a colorless crystalline material. Analytical data obtained on this material and I. R. spectra (Table 1) as well as the mild alkaline hydrolysis to chloramphenicol suggested the chloramphenicol-butyrate or isobutyrate structure. The N. M. R. spectrum furthermore suggested the structure of chloramphenicol-3-isobutyrate. This conclusion is based on the observation that the spectrum contained a doublet at δ 1.0 ppm of area 6 assigned to the isopropyl group. In addition, the absorption peak of the 2H at C-3, which is present at δ 3.5 in the spectrum of chloramphenicol, has shifted to δ 4.2, suggesting the presence of the ester grouping at C-3.

However, the mass spectrum of this preparation indicated that **V** contained two compounds. The main component, compound **Va** showed M+1 isotopic clusters at 393, 395, 397 consistent with the chloramphenicol isobutyrate structure. The peaks at 291 [$M-CH_2OCOCH(CH_3)_2$] and 240 (fragment F, Fig. 3) mass units indicate the presence of

the isobutyrate group at C-3. The peak at 152 mass units indicate that the secondary hydroxyl group is free. The minor component, compound **Vb** showed M+1 ionic cluster peaks at 407, 409, 411 in agreement with chloramphenicol isovalerate structure. Peaks at 291 [M-$CH_2OCOCH_2CH(CH_3)_2$] and 254 (fragment G, Fig. 3) mass units indicate the presence of the ester grouping at C-3. The chloramphenicol isovalerate structure is consistent with the N. M. R. data of the mixture. However, a definite structural assignment cannot be made at this time.

In some respects the acylation of chloramphenicol by *S. coelicolor* resembles the acetylation of this antibiotic by R factor carrying *Escherichia coli* strains[4,5]. It might also be noted that all five chloramphenicol acylates (**II, III, IV, Va, Vb**) are inactive *in vitro* against *Klebsiella pneumoniae* and other organisms sensitive to chloramphenicol. However, they are as effective as chloramphenicol in protecting mice infected with *K. pneumoniae*.

A. D. ARGOUDELIS
J. H. COATS

Research Laboratories,
The Upjohn Company
Kalamazoo, Michigan 49001
U. S. A.

(Received January 11, 1971)

References

1) COATS, J. H. & A. D. ARGOUDELIS : Microbial transformation of antibiotics. IV. Phosphorylation of clindamycin by *Streptomyces coelicolor* MÜLLER. (To be published)
2) ARGOUDELIS, A. D.; J. H. COATS, D. J. MASON & O. K. SEBEK : Microbial transformation of antibiotics. III. Conversion of clindamycin to 1′-demethylclindamycin and clindamycin sulfoxide by *Streptomyces* species. J. Antibiotics 22 : 341～343, 1969
3) ARGOUDELIS, A. D. & J. H. COATS : Microbial transformation of antibiotics. V. Clindamycin ribonucleotides. (To be published)
4) SHAW, W. V. : The enzymatic acetylation of chloramphenicol by extracts of R factor-resistant *Escherichia coli*. J. Biol. Chem. 242 : 687～693, 1967
5) SUZUKI, Y. & S. OKAMOTO : The enzymatic acetylation of chloramphenicol by the multiple drug-resistant *Escherichia coli* carrying R factor. J. Biol. Chem. 242 : 4722～4730, 1967