Communication to the editors

MICROBIAL TRANSFORMATION OF ANTIBIOTICS. II

PHOSPHORYLATION OF LINCOMYCIN BY *STREPTOMYCES* SPECIES

Sir:

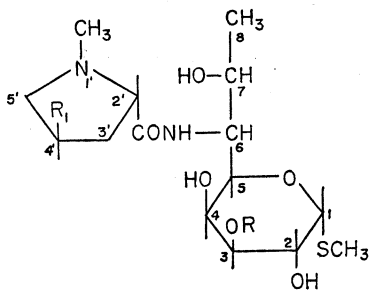
In the preceding paper in this series[1] we reported on the transformation of lincomycin (I) to lincomycin sulfoxide and 1-demethylthio-1-hydroxylincomycin by *Streptomyces lincolnensis*. This communication describes studies related to the microbial transformation of lincomycin to lincomycin-3-phosphate (II) (Fig. 1).

Several streptomycete species were found to have the ability to inactivate lincomycin. For example, *Streptomyces rochei*, grown in a synthetic medium, completely inactivated 50 mcg/ml of lincomycin in less than 48 hours. With the use of ^{35}S-lincomycin it was found that the antibiotic was transformed to a single compound. As shown in Fig. 2, immediately after addition of radio-active lincomycin to the fermentation a single *bio*active and radioactive spot was observed. After 24 hours, more than half of the radioactive lincomycin added had been transformed to a *bio*inactive compound. After 48 hours almost all of the radioactivity was in a single peak, well separated from lincomycin.

The inactivated lincomycin was extracted from the beer efficiently by adsorption on carbon followed by elution with aqueous acetone. It was purified by counter double current distribution and countercurrent distribution using 1-butanol - water (1 : 1 v/v) as the solvent system.

The molecular formula of the isolated compound ($C_{18}H_{35}N_2O_9SP$) plus

Fig. 1.



I: R=H; $R_1=CH_2CH_2CH_3$

II: R=P(=O)(OH)-OH; $R_1=CH_2CH_2CH_3$

Fig. 3. Isolated lincomycin phosphate

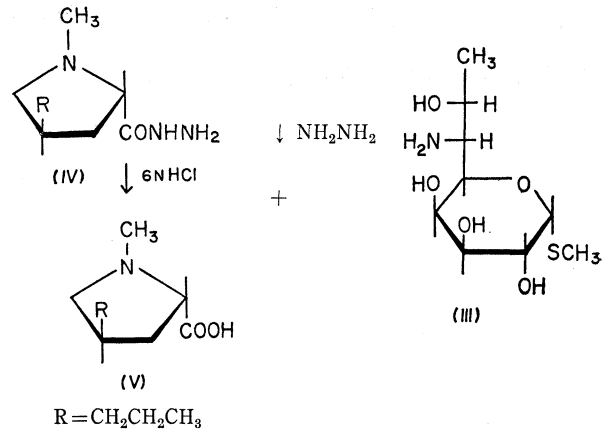
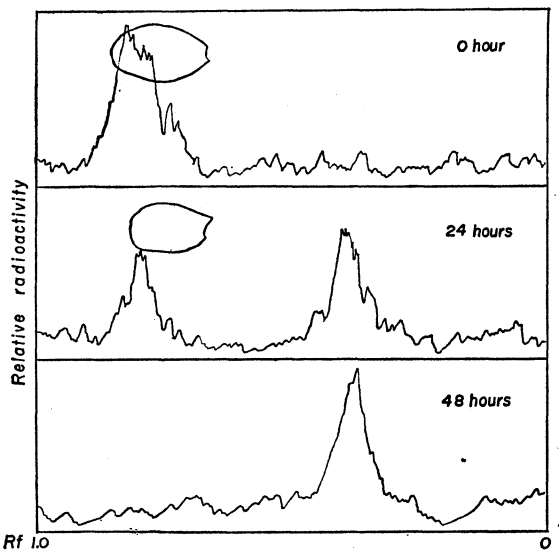


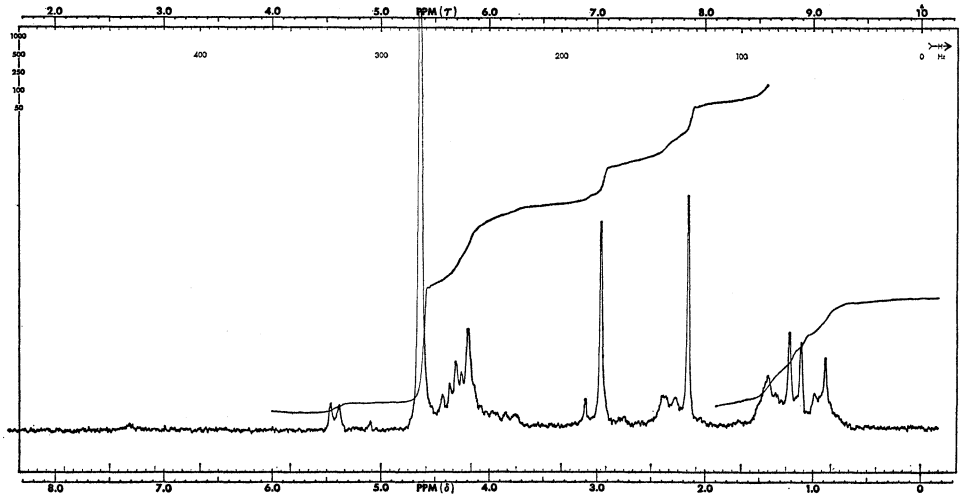
Fig. 2. Bio-radiogram[1,2] of fermentation beer of *S. rochei* containing [^{35}S]-lincomycin



1) Solvent: 1-Butanol - water (84 : 16 v/v) containing 0.2 % of piperidine

2) Peaks indicate radioactivity while circles show zones of *bio*acitivity *vs. Sarcina lutea*.

Fig. 4. Nuclear magnetic resonance spectrum of lincomycin-3-phosphate.



the specific rotation ($[\alpha]_D^{25}$ +127°, *c* 0.7 water) and the infrared spectrum indicated a lincomycin-related material. The lack of *in vitro* activity, the presence of one phosphorous atom per molecule and the production of lincomycin by treatment with alkaline phosphatase indicates that P is present as part of a phosphate ester grouping. This conclusion is in agreement with potentiometric titration data.

Furthermore, hydrazinolysis[2] of the obtained material afforded α-methyl thiolincosaminide (III) and the hydrazide of 1-methyl-4-propyl-L-proline (IV) which was transformed to the crystalline hydrochloride of V by acid hydrolysis. These results are in agreement with the postulation of a lincomycin phosphate ester structure for the *bio*-inactive material.

The nuclear magnetic resonance spectrum* (Fig. 4) of the phosphorylated lincomycin was identical to the spectrum of lincomycin in all areas except in the carbinolhydrogen region. This indicates that the phosphate ester group is attached to carbon 2, 3, 4, or 7 of the aminosugar part of the molecule. The microbiologically produced lincomycin phosphate was compared to lincomycin-2-phosphate and lincomycin-7-phosphate esters (prepared by Dr. W. MOROZOWICH of The Upjohn Company) and was found to be different from both. This leaves carbon 3 or 4 of the sugar moiety of the lincomycin molecule as the possible point of attachment of the phosphate group.

We propose the lincomycin-3-phosphate (II) structure on the basis of periodate oxidation. In this oxidation, lincomycin is expected to consume 3 moles of periodate, one for oxidation on the sulfur and two for the oxidation of the two vicinal glycol groupings. Similarly lincomycin-2-phosphate and lincomycin-4-phosphate are expected to consume 2 moles of periodate. Lincomycin-3-phosphate should consume only one mole of periodate (for oxidation of the sulfur) since the presence of the phosphate group on C-3 eliminates glycol grouping in the molecule. Actually, when lincomycin, lincomycin-2-phosphate, and the isolated lincomycin-phosphate were oxidized by periodate under identical conditions, they consumed 3.5, 2.1 and 1.1 moles of periodate respectively. These data, therefore, suggest that the isolated lincomycin-phosphate is indeed lincomycin-3-phosphate.

Lincomycin-3-phosphate is inactive *in vitro* against several organisms including *S. aureus*. However, this compound was found to protect *S. aureus* infected mice with a CD_{50}[3] of *ca.* 30 mg/kg when it was administered subcutaneously.

* 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 deuterium oxide.

It is of interest that in addition to lincomycin, other lincomycin-related antibiotics such as 4′-depropyl-4′-ethyllincomycin (U-21,699)[4], S-demethyl-S-ethyllincomycin (U-11,921)[5], and 1′-demethyllincomycin (U-11,973)[6] as well as celesticetin[7] were also phosphorylated under conditions similar to those used for lincomycin.

Acknowledgements

The authors are greateful to Mr. FOREST MAC KELLAR for n.m.r. data and to Mr. K. J. GEIPEL and W. A. MURPHY for technical assistance.

A. D. ARGOUDELIS
J. H. COATS

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

(Received May 2, 1969)

References

1) ARGOUDELIS, A. D. & D. J. MASON: Microbial transformation of antibiotics. I. Production of lincomycin sulfoxide and 1-demethylthio-1-hydroxylincomycin by *S. lincolnensis*. J. Antibiotics 22 : 289～291, 1969.
2) HOEKSEMA, H.; B. BANNISTER, R. D. BIRKENMEYER, F. KAGAN, B. J. MAGERLEIN, F. A. MACKELLAR, W. SCHROEDER, G. SLOMP & R. R. HERR: Chemical studies on lincomycin. I. The structure of lincomycin. J. Am. Chem. Soc. 86 : 4223～4224, 1964.
3) SPEARMAN, C. & G. KARBER in D. J. FINNEY (Ed.): "Statistical methods in biological assay", 2 nd Ed. pp. 524～530. Hafner Publishing Co., N. Y., N. Y.
4) ARGOUDELIS, A. D.; J. A. FOX & T. E. EBLE: U-21,699, a new lincomycin-related antibiotic. Biochemistry 4 : 698～703, 1965.
5) ARGOUDELIS, A. D. & D. J. MASON: Studies on the biosynthesis of lincomycin. I. Antibiotic U-11,921, an S-ethyl homolog of lincomycin. Biochemistry 4 : 704～709, 1965.
6) ARGOUDELIS, A. D.; J. A. FOX & D. J. MASON: Studies on the biosynthesis of lincomycin. II. Antibiotic U-11,973, N-demethyllincomycin. Biochemistry 4 : 710～713, 1965.
7) HOEKSEMA, H.: Celesticetin. V. The structure of celesticetin. J. Am. Chem. Soc. 90 : 755～757, 1968.