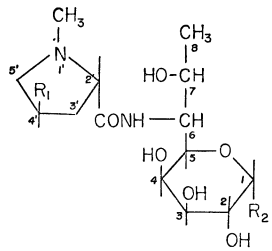
MICROBIAL TRANSFORMATION OF ANTIBIOTICS. I

PRODUCTION OF LINCOMYCIN SULFOXIDE AND 1-DEMETHYLTHIO-1-HYDROXYLINCOMYCIN BY *S. LINCOLNENSIS*

Sir:

Streptomyces lincolnensis var. *lincolnensis* produces lincomycin (I) and 4′-depropyl-4′-ethyllincomycin (U-21,699) (II) in six-day fermentations as described by MASON *et al.*[1]

Fig. 1



I $R_1=CH_2CH_2CH_3$; $R_2=-SCH_3$

II $R_1=CH_2CH_3$; $R_2=-SCH_3$

III $R_1=CH_2CH_2CH_3$; $R_2=-S-CH_3$ (→O)

IV $R_1=CH_2CH_2CH_3$; $R_2=-OH$

Extension of the fermentation period to 12 days resulted in the production of two additional biologically active compounds with very similar Rf values in several TLC and paper chromatographic systems. Since these new activities had a biological spectrum similar to that of lincomycin we suspected that both compounds were formed by transformation of lincomycin itself.

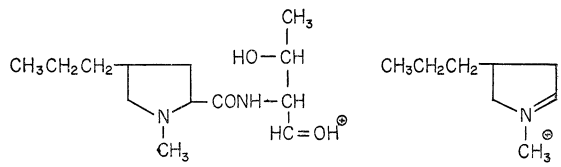
To prove this assumption, radioactive lincomycin was added to a 6-day old lincomycin fermentation. Paper chromatography of the fermentation beer after 6 additional days established that some of the lincomycin had indeed been transformed to compounds with Rf values identical to those of the two new activities.

Lincomycin, 4′-depropyl-4′-ethyllincomycin and the two new compounds were extracted from filtered broth by liquid ion exchangers. Lincomycin and the other lincomycin-related antibiotics formed salts with sodium dinonylnaphthalene sulfonate which were soluble in organic solvents. These salts could readily be extracted into methylene chloride. The methylene chloride extracts were then mixed with a solution of trioctyl ethyl ammonium chloride in methylene chloride. This treatment resulted in the formation of the hydrochloride salts of the antibiotics which were thus transferred into water.

The two new compounds were separated from lincomycin by counter double current distribution using 1-butanol – water (1 : 1) as the solvent system. The two new activities were separated from each other by conventional countercurrent distribution using the same solvent system.

The first of these compounds, isolated as the hydrochloride salt, $C_{18}H_{34}N_2O_7S\cdot HCl\cdot H_2O$, had an infrared spectrum similar to that of lincomycin [-OH, NH absorption and amide type absorption at *ca.* 1680 cm^{-1}(amide I) and 1565 cm^{-1} (amide II)]. This material was identified as lincomycin sulfoxide (III) by comparison of its nuclear magnetic resonance* spectrum with that of lincomycin (Fig. 3). The n. m. r. spectrum of this compound differs from the spectrum of lincomycin mainly in two areas. The singlet at δ 2.16 (3H) assigned to the $-SCH_3$ group of lincomycin shifts downfield to δ 2.85 in the spectrum of the sulfoxide. Furthermore the doublet at δ 5.41 (1H) assigned to the anomeric hydrogen of lincomycin shifts to δ 4.83 in the spectrum of the sulfoxide. These results show that the sulfur of the methylthio glycosidic group has been oxidized[2]. Further confirmation of the lincomycin sulfoxide structure was obtained by mass spectra. Lincomycin exhibits mass ion peaks at m/e 406 (molecular ion), 359 ($M-SCH_3$), 257 due to ion **V** (Fig. 2) and 126 due to ion **VI** (Fig. 2)[3]. The spectrum of the new activity showed a molecular ion peak at m/e 422 and also peaks at 359 ($M-SOCH_3$), 257 and 126 due to the same ions present in the spectrum of lincomycin.

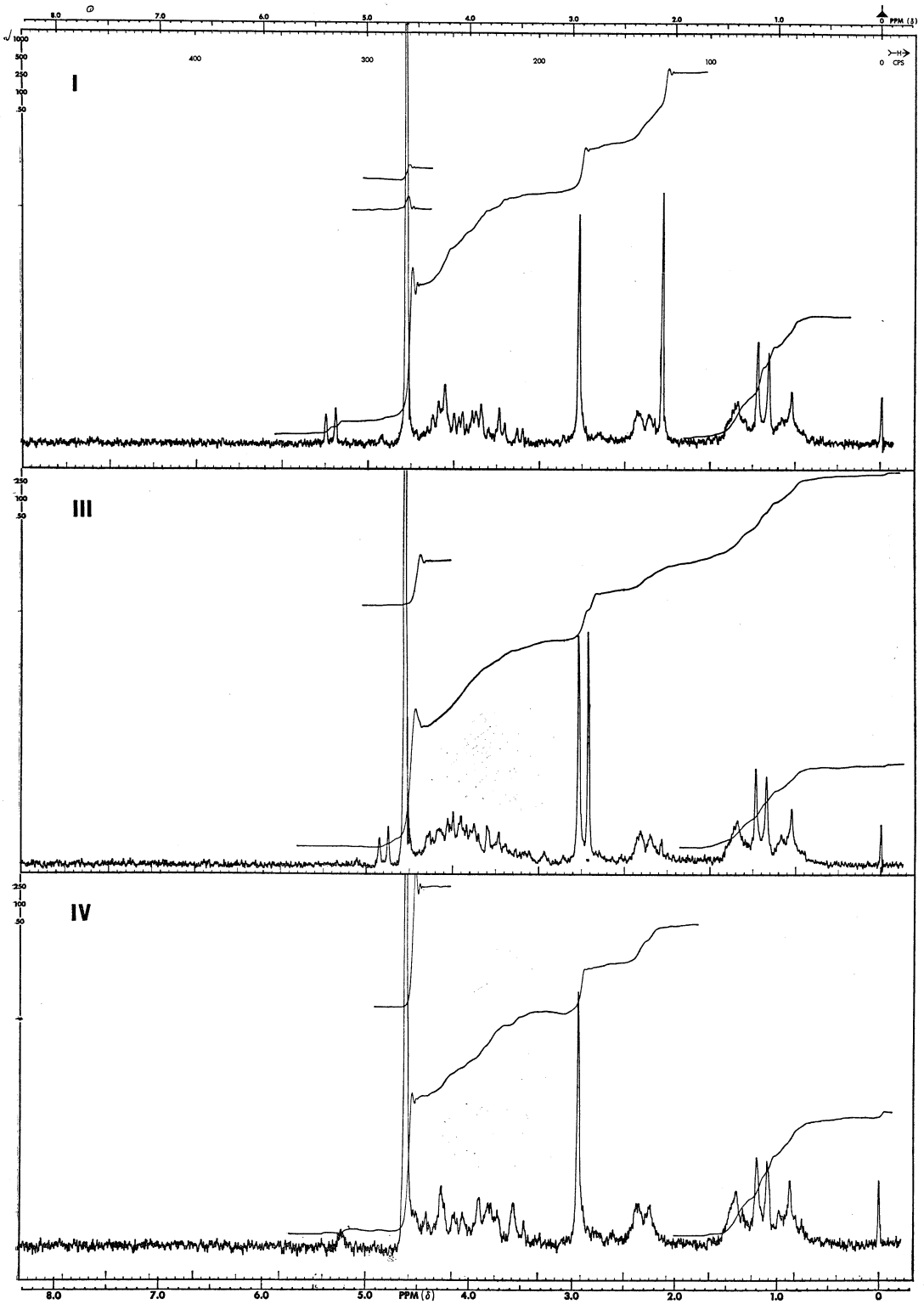
Fig. 2



V **VI**

* 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.

Fig. 3. Nuclear magnetic resonance spectra of lincomycin (I), lincomycin sulfoxide (III), and 1-demethylthio-1-hydroxylincomycin (IV)



The second of the new bioactive compounds, isolated as the hydrochloride salt, has been identified as 1-demethylthio-1-hydroxylincomycin (**IV**). This compound had a lincomycin type infrared spectrum but did not contain sulfur ($C_{17}H_{32}N_2O_7 \cdot HCl \cdot H_2O$). Furthermore, its n. m. r. spectrum (Fig. 3) did not show the presence of the $-SCH_3$ group of lincomycin. A mixture of α and β anomers is indicated by the broad peak at δ 5.02 due to the anomeric hydrogen. The mass spectrum showed a molecular ion peak at m/e 376 and peaks at 358 ($M-H_2O$), 257 and 126 due to ions **V** and **VI** which are also present in the spectrum of lincomycin.

Lincomycin sulfoxide and 1-demethylthio-1-hydroxylincomycin obtained by fermentation were found identical to authentic samples prepared chemically by Mr. R. BIRKENMEYER and Dr. B. BANNISTER of The Upjohn Company.

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