# MICROBIAL TRANSFORMATION OF ANTIBIOTICS. III

## CONVERSION OF CLINDAMYCIN TO 1′-DEMETHYLCLINDAMYCIN AND CLINDAMYCIN SULFOXIDE BY *STREPTOMYCES* SPECIES

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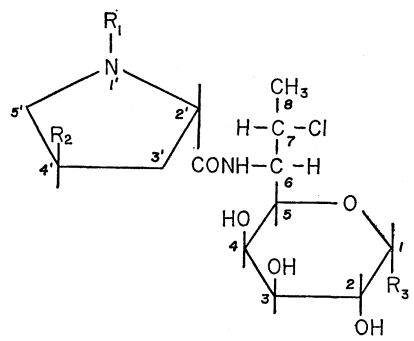
1′-Demethylclindamycin was produced by N-demethylation when clindamycin was added to fermentations of *Streptomyces punipalus*. In contrast, addition of clindamycin to fermentations of *Streptomyces armentosus* resulted primarily in the production of clindamycin sulfoxide. Several other species of streptomycetes as well as several species of fungi were also found to convert clindamycin to either 1′-demethylclindamycin, clindamycin sulfoxide, or a mixture of both compounds.

Clindamycin (I) (Fig. 1) is a new clinically useful antibacterial agent produced by chlorination of lincomycin[1]. The 7-hydroxy group of lincomycin is replaced by chlorine with inversion of the configuration at the 7-carbon. Similarly 1′-demethylclindamycin (II) is produced by chlorination of 1′-demethyllincomycin. As shown in Table 1, clindamycin is four times as active *in vitro vs. Sarcina lutea* as lincomycin. 1′-Demethyllincomycin is about one twentieth as active as lincomycin *in vitro*. 1′-Demethylclindamycin, however, is twice as active as clindamycin. In addition to its antibacterial activity, 1′-demethylclindamycin has been found to be highly effective in treating *Plasmodium berghei* infections in mice[2]. This antimalarial activity has not been seen previously in the lincomycin family of antibiotics.

Fig. 1.



I $R_1=CH_3$; $R_2=CH_2CH_2CH_3$; $R_3=SCH_3$
II $R_1=H$; $R_2=CH_2CH_2CH_3$; $R_3=SCH_3$
III $R_1=CH_3$; $R_2=CH_2CH_2CH_3$; $R_3=SCH_3 \rightarrow O$

Table 1. Relative *in vitro* activity against *S. lutea*

| | Relative activity |
|---|---|
| Lincomycin | 1 |
| 1′-Demethyllincomycin | 0.05 |
| Lincomycin sulfoxide | 0.01 |
| Clindamycin | 4 |
| 1′-Demethylclindamycin | 8 |
| Clindamycin sulfoxide | 1 |

In previous communications in this series we reported the transformation of lincomycin to lincomycin sulfoxide and 1-demethylthio-1-hydroxylincomycin by

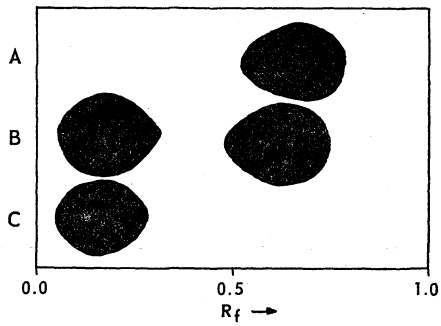
*Streptomyces lincolnensis*[3] and the phosphorylation of lincomycin to lincomycin-3-phosphate by *Streptomyces rochei*[4]. Since 1′-demethylclindamycin is a potent antibacterial agent, its production by microbial transformation of clindamycin was attempted. The present paper discusses the results of these studies.

## Discussion

Studies on the biosynthesis of lincomycin have shown that the $-NCH_3$, the $-SCH_3$ and the terminal methyl group of the *n*-propyl side chain of the amino acid moiety originate from $C_1$ donors by transmethylation[6]. The biological transformation of clindamycin to 1′-demethylclindamycin requires selective demethylation of the $-NCH_3$ group without any effect on the two other groups ($-SCH_3$, $-CCH_3$) of similar bio-origin. Examples of selective demethylation by biological systems have been reported previously by others. Addition of colchicine and thiocolchicine to cultures of *Streptomyces griseus* resulted in demethylation of these compounds[7]. It is of interest to note that demethylation of thiocolchicine (which contains both $-OCH_3$ and $-SCH_3$ groups) occurs by removal of a methyl group from the ether function but not from the sulfur. N-Demethylerythromycin was found to be the main *in vivo* metabolite of erythromycin and results from selective N-demethylation of the antibiotic[8]. The $-OCH_3$ and $-CCH_3$, groups of erythromycin which, along with the dimethylamino group, originate by transmethylation, were not affected.

In our studies, it was found that when clindamycin was added at a level of 500 mcg per ml in fermentation of *Streptomyces punipalus* the antibiotic was transformed to a single bioactive compound with an Rf value identical to that of 1′-demethylclindamycin (Fig. 2). This material was conclusively identified as 1′-demethylclindamycin by mass spectra. The mass spectrum (Fig. 3) of the microbiologically-produced 1′-demethylclindamycin ($C_{17}H_{31}N_2O_5SCl$) shows molecular ion peaks at m/e 410, 412 (relative intensity of peaks 3 : 1

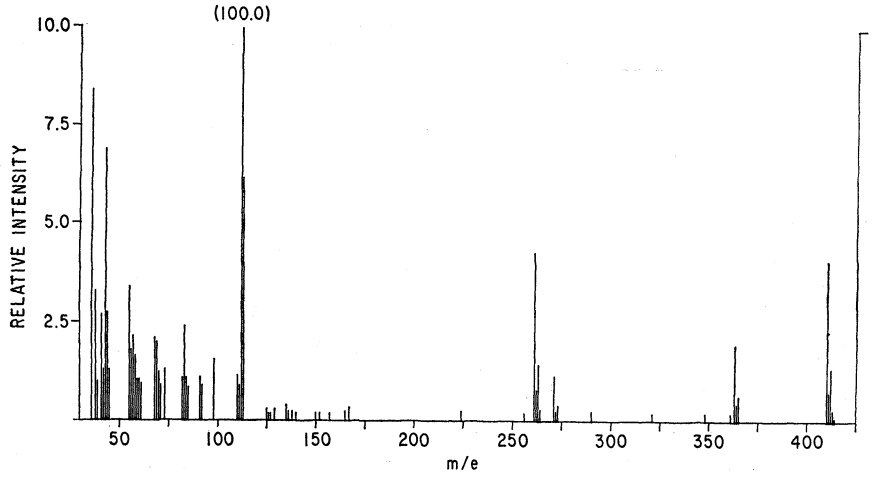
Fig. 2. Thin-layer chromatogram




A. Clindamycin
B. *S. punipalus* fermentation at harvest time
C. 1′-Demethylclindamycin

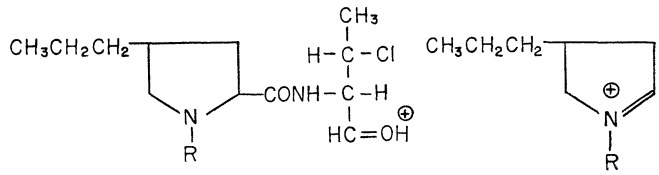
[Silica gel; Ethyl acetate - acetone - water (8 : 5 : 1 v/v)]

Fig. 3. Mass spectrum of 1′-demethylclindamycin.



as expected for this chlorine containing antibiotic); at 363, 365 due to M-$SCH_3$ ion; at 261, 263 due to ion **IV**a (Fig. 4) and the base peak at 112 due to ion **V**a resulting from the propylproline moiety[9].

Fig. 4.




IVa R=H Va R=H
IVb R=$CH_3$ Vb R=$CH_3$

Although *S. punipalus* transforms clindamycin principally to 1′-demethylclindamycin, trace amounts of a bioactive material different from either clindamycin or 1′-demethylclindamycin have been occasionally detected in cultures of this organism grown in the presence of clindamycin. This new bioactive material is the main transformation product when clindamycin is incubated with another streptomycete, *Streptomyces armentosus*.

The compound, isolated as the crystalline hydrochloride salt, $C_{18}H_{33}N_2O_6ClS \cdot HCl \cdot H_2O$, has an infrared spectrum similar to that of clindamycin. This material was identified as

Fig. 5. Mass spectrum of clindamycin sulfoxide.

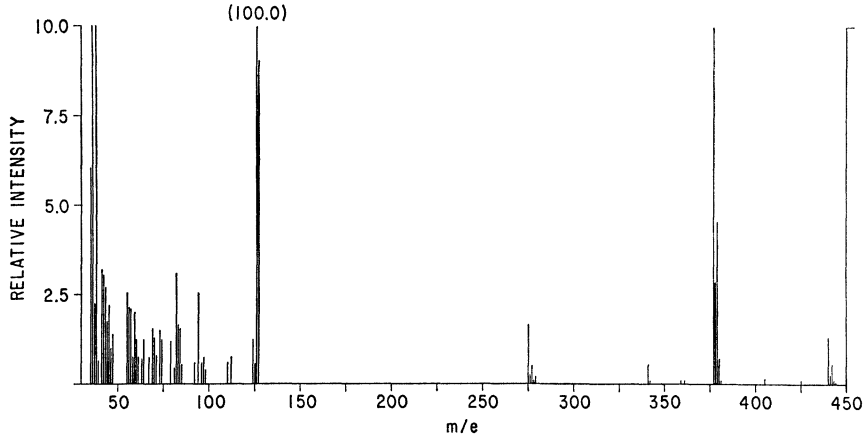
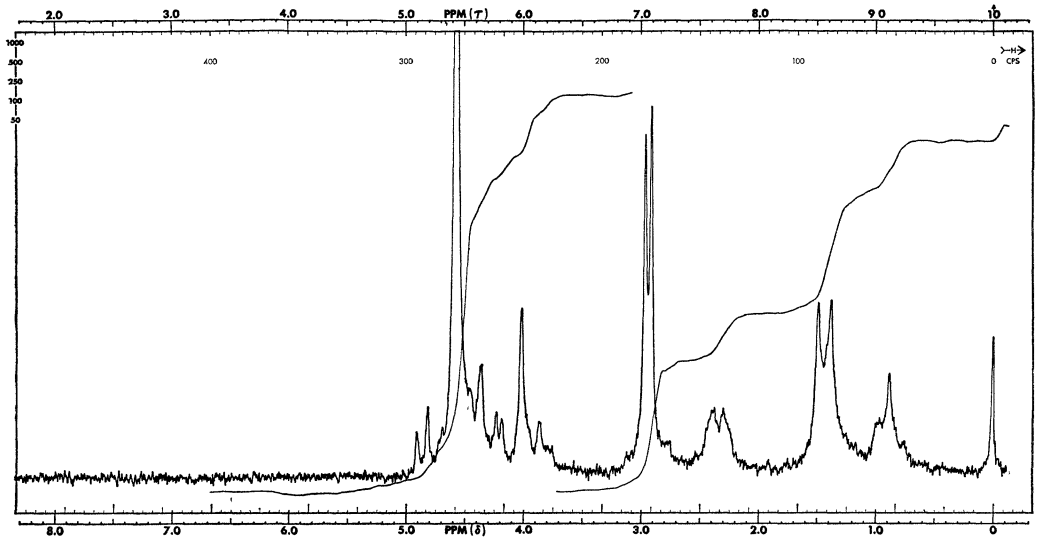



Fig. 6. Nuclear magnetic resonance spectrum of clindamycin sulfoxide.



clindamycin sulfoxide by n.m.r. and mass spectroscopy. The n.m.r. spectrum (Fig. 6) differs from the spectrum of clindamycin mainly in two areas. The singlet at $\delta$ 2.16 (3H) assigned to the $-SCH_3$ group of clindamycin shifts downfield to $\delta$ 2.88 in the spectrum of the sulfoxide. Furthermore, the doublet at $\delta$ 5.41 (1H) assigned to the anomeric hydrogen of clindamycin shifts to $\delta$ 4.83 in the spectrum of clindamycin sulfoxide. These results show oxidation of the sulfur[10]. Further confirmation of the clindamycin sulfoxide structure was obtained by mass spectroscopy. The mass spectrum (Fig. 5) showed molecular ion peaks at m/e 440, 442 and also peaks at 377, 379 ($M-SOCH_3$), at 275, 277 due to ion **IV**b (Fig. 4) and the base peak at 126 due to ion **V**b.

In addition to *S. punipalus* and *S. armentosus*, several other species of streptomycetes as well as fungi were found to transform clindamycin to either 1′-demethylclindamycin or clindamycin sulfoxide or a mixture of these two compounds. The transformation of clindamycin to 1′-demethylclindamycin, *i. e.* the N-demethylation reaction, can be postulated to occur either through transmethylation or oxidative demethylation. Both mechanisms have been described in the literature.[11,12,13] The latter mechanism, which involves oxidation of the methyl group, appears more attractive since several of the species which have the ability to N-demethylate clindamycin also have the ability to transform clindamycin to clindamycin sulfoxide.

## Experimental

I. General

Antibiotic titers were measured by disc plate activity using *S. lutea* as the assay organism[5]. Fermentation beers were analyzed by thin-layer chromatography on silica gel using ethyl acetate – acetone – water (8 : 5 : 1 v/v) as the solvent system.

II. Transformation of Clindamycin to 1′-Demethylclindamycin

A. Fermentation Procedures: Seed cultures of *S. punipalus* were prepared in a medium consisting of glucose monohydrate (Cerelose), 25 g/liter and Pharmamedia, 25 g/liter. The cultures were incubated at 28°C for 72 hours on a rotary shaker. A fermentation medium consisting of dextrin, 30 g/liter; black strap molasses 20 g/liter; fish meal, 15 g/liter and Pharmamedia, 15 g/liter, was inoculated with 72-hour seed culture at a rate of 5 percent (v/v). The cultures were incubated at 28°C on a rotary shaker (250 rpm, 6 cm stroke). Clindamycin hydrochloride (500 mcg/ml) was added after the fermentation had progressed 48 hours. Beers were harvested after a total fermentation time of 72 hours. A thin-layer chromatogram of a typical *S. punipalus* fermentation, at harvest time, is presented in Fig. 2.

B. Extraction of Antibiotic Activities from Fermentation Beer: Fermentation beer (*ca.* 10 liters) was filtered at harvest pH using filter aid. The mycelial cake was washed with water and discarded. The filtered beer and the wash were pooled and passed over a column prepared from 350 g of Amberlite XAD-2 (Rohm and Haas Co., Philadelphia, Pa.). The column was then washed with 2 liters of water. Both the spent beer and the aqueous wash were inactive when tested against *S. lutea* and were discarded. The *bio*activities were eluted with 2 liters of a mixture of methyl ethyl ketone – water (95 : 5 v/v). The eluate was concentrated to dryness; yield 7.0 g. Thin-layer chromatography of this material showed clindamycin and 1′-demethylclindamycin as the only *bio*active components.

C. Separation of Clindamycin from 1′-Demethylclindamycin. Silica Gel Chromatography; A column was prepared from 600 g of silica gel (Merck-Darmstadt, 7734) packed in the solvent system consisting of chloroform – methanol (6 : 1 v/v). Six g of material obtained as described in the previous section, was chromatographed over the column using the above solvent system. Fractions were analyzed by thin-layer chromatography. Clindamycin was eluted first followed by 1′-demethylclindamycin. Eluates containing

1′-demethylclindamycin were combined and concentrated to dryness. The residue was dissolved in methylene chloride and this solution was mixed with Skellysolve B. The precipitated material was isolated by filtration and dried; yield 350 mg. This material was identical to an authentic sample of 1′-demethylclindamycin. The mass spectrum of the microbiologically produced 1′-demethylclindamycin is presented in Fig. 3.

III. Transformation of Clindamycin to Clindamycin Sulfoxide

A. Fermentation Procedures: Seed cultures of *S. armentosus* were prepared in a medium identical to that used for preparation of seed cultures of *S. punipalus*. (See I-A above.) The fermentation medium consisting of starch 30 g/liter; black strap molasses, 20 g/liter; Pharmamedia 15 g/liter; fish meal, 15 g/liter and lard oil 5 ml/liter was inoculated with the 72-hour seed culture at a rate of 5 percent (v/v). The cultures were incubated at 28°C on a rotary shaker (250 rpm, 6 cm stroke). Clindamycin hydrochloride (500 mcg/ml) was added after the growth had progressed 48 hours. Beers were harvested after a total incubation time of 120 hours.

B. Extraction of Antibiotic Activities from Fermentation Beers; Fermentation beer (*ca.* 4 liters) was filtered using filter aid and the cake washed with water. The combined clear beer-wash was adjusted to pH 10.0 with aqueous sodium hydroxide and extracted three times with 1,600 ml-portions of methylene chloride. The methylene chloride extract was concentrated to dryness to give 1.0 g of material which was found to contain clindamycin and clindamycin sulfoxide as the main bioactive components. Thin-layer chromatography also showed the presence of trace amounts of 1′-demethylclindamycin.

C. Separation of Clindamycin Sulfoxide from Clindamycin by Countercurrent Distribution: The mixture of clindamycin sulfoxide and clindamycin was dissolved in the lower phase of a system consisting of equal volumes of 1-butanol and water. The solution was adjusted to pH 4.0 with 1 N hydrochloric acid. Upper phase was added and the hydrochloride salts were distributed in a countercurrent distribution apparatus. After 500 transfers, the distribution was analyzed by thin-layer chromatography and determination of solids. Fractions containing clindamycin sulfoxide hydrochloride (K=0.14) were concentrated to dryness. Trituration of the residue with acetone afforded crystalline clindamycin sulfoxide hydrochloride; yield 440 mg.

Anal. Calcd. for $C_{18}H_{33}N_2O_6SCl \cdot H_2O \cdot HCl$: C 43.63, H 7.27, N 5.66, S 6.46, Cl 14.34.
Found: C 42.62, H 7.38, N 5.91, S 6.32, Cl 14.25.

The infrared spectrum (in Nujol mull) showed absorptions at 3400~3200 $cm^{-1}$ (OH, NH); 2720~2570 $cm^{-1}$ ($-\overset{+}{N}CH_3$); 1687 $cm^{-1}$ (amide I) and 1574 $cm^{-1}$ (amide II). The mass spectrum of clindamycin sulfoxide is shown in Fig. 5. The n.m.r. spectrum* is shown in Fig. 6.

Clindamycin hydrochloride (100 mg) isolated from fractions with K value of 0.32, was found to be contaminated with small amounts of 1′-demethylclindamycin hydrochloride.

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* 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.

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