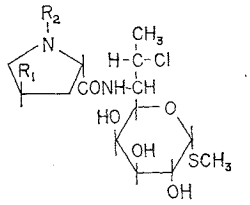
MICROBIAL TRANSFORMATION OF ANTIBIOTICS VII. HYDROXYMETHYLATION OF N-DEMETHYLCLINDAMYCIN

Sir:

We have reported[1] that several streptomycetes transform clindamycin (I, Fig. 1) to N-demethylclindamycin (II, Fig. 1).

Fig. 1.



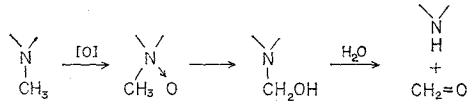
I $R_1 = CH_2CH_2CH_3$ $R_2 = CH_3$

II $R_1 = CH_2CH_2CH_3$ $R_2 = H$

III $R_1 = CH_2CH_2CH_2CH_2CH_3$ $R_2 = H$

The mechanism of this enzymatic demethylation is not known. However, enzymatic N-demethylation of tertiary amines is believed[2,3] to proceed by the steps outlined in Fig. 2.

Fig. 2.

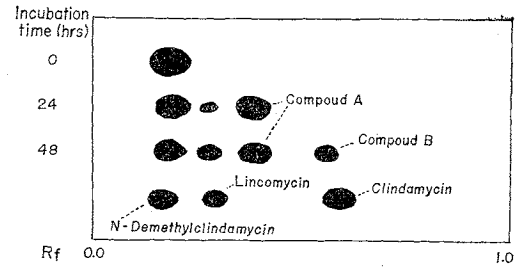


We were interested in determining whether the methylation of N-demethylclindamycin to clindamycin could also be carried out by streptomycetes. We selected *Streptomyces lincolnensis* for these studies, since this organism contains[4] efficient methylating systems.

N-Demethylclindamycin was added to 24-hour cultures of *S. lincolnensis*[5] at levels of 50~100 mcg/ml. As shown in Fig. 3, immediately after the addition of N-demthylclindamycin (0 time) this antibiotic was the only bioactive compound in the fermentation broth. Twenty four hours after addition of the compound, part of the N-demethylclindamycin had been transformed to a new bioactive material, compound A. At that time the fermentation broth also contained lincomycin normally produced by the culture. Forty eight hours after addition, tlc showed

Fig. 3. Thin-layer chromatography* of fermentations of *Streptomyces lincolnensis* containing N-demethylclindamycin.

* Silica Gel G: Ethyl acetate-acetone-water (8:5:1 v/v). Antibiotics were detected by bioautography on *S. lutea*-seeded agar.



the presence of N-demethylclindamycin, lincomycin, compound A, and a new bioactive material, compound B. *S. lincolnensis* growing in the absence of N-demethylclindamycin, produced lincomycin only. Since less compound A was present in the 48-hour sample than in the 24-hour sample it was assumed that both compounds A and B are biotransformation products of N-demethylclindamycin and that A is formed first and slowly transformed to compound B.

4′-Depropyl-4′-pentyl-N-demethylclindamycin (III, Fig. 1) was also transformed by *S. lincolnensis*. Clindamycin, however, was not effected at all by this organism. This suggested the imino group of propyl proline moiety of N-demethylclindamycin as the site of biotransformation.

We directed our efforts to the isolation of compound A since this material was the main bioconversion product of N-demethylclindamycin. The activities present in the fermentation broth were adsorbed on Amberlite XAD-2 and eluted using 95% aqueous methanol. Compound A is very unstable and is converted to N-demethylclindamycin and small amounts of compound B when subjected to countercurrent distribution, silica gel, and partition or Sephadex chromatographies. When CM-Sephadex, a cationic exchanger was used, N-demethylclindamycin and lincomycin were adsorbed, while compound A passed through the column. This compound, however, was partially converted to N-demethylclindamycin and compound B during the recovery process. The behavior of compound A suggested a neutral or weakly basic compound which

Fig. 4.

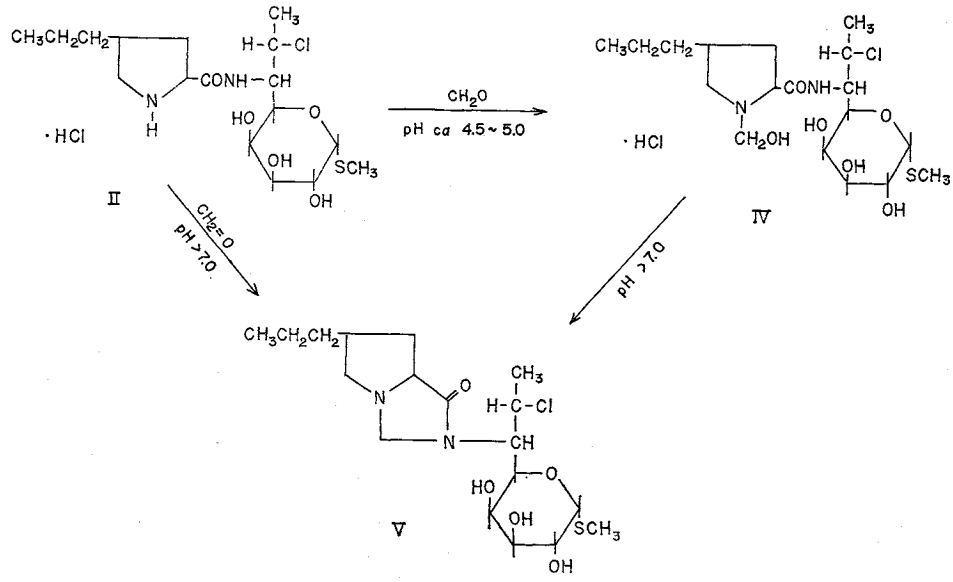
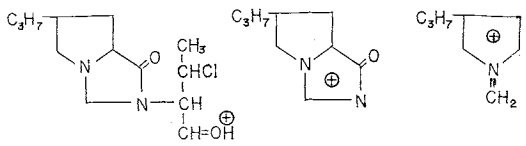


Fig. 5.



could be degraded to N-demethylclindamycin under mild conditions. N-Demethyl-N-hydroxymethylclindamycin (**IV**, Fig. 4), is such a compound. This material is expected to be unstable and to be easibly degraded to N-demethylclindamycin and formaldehyde. One also would expect that hydroxymethylation of the secondary amino-group would decrease the basic properties of N-demethylclindamycin[6]. This is in agreement with the fact that compound A was not adsorbed by CM-Sephadex. Compound A under isolation conditions was transformed not only to N-demethylclindamycin but to compound B. Therefore, any structure proposed for compound A should take into account this transformation.

We proceeded to synthesize N-demethyl-N-hydroxymethylclindamycin. Mixing an aqueous solution of N-demethylclindamycin hydrochloride with formalin at room temperature gave a compound with Rf values greater than those of N-demethylclindamycin and identical to those of compound A. The product of the reaction, assigned structure **IV**, was isolated as a colorless, amorphous, highly hygroscopic material, showing secondary amide carbonyl absorption at 1680, and 1500 cm^{-} in the IR spectrum. This material was highly unstable and decomposed to N-demethylclindamycin and formaldehyde. Mass spectra were not helpful in establishing the structure. The NMR spectrum on the other hand, shows the presence of all the groupings present in N-demethylclindamycin and in addition an absorption peak at *ca* δ, 5.2 assigned to the N-hydroxymethyl group.

When the reaction of N-demethylclindamycin and formaldhyde was carried out at pH greater than 7.0, the product of the reaction was a new bioactive compound which had Rf values identical to those of compound B. In addition when solutions of N-demethyl-N-hydroxymethylclindamycin were adjusted to pH greater than 7.0 the new compound was formed almost quantitatively. This compound, isolated crystalline, was found to be very stable and to have structure **V** (Fig. 4).

Analytical data agree with the molecular formula of $C_{18}H_{31}ClN_2O_5S$ for **V**. Potentiometric titration in water shows the presence of one weakly basic group with pKa' of 4.1 (Eq. weight found, 418). The IR spectrum shows the absence of the amide II

band (at *ca* 1,500 cm^{-1}) common to all lincosaminides indicating a tertiary amide carbonyl. The mass spectrum contains molecular ion peak at *m/e* 422,1616. (Theor. mol. weight, 422,1642). The spectrum also shows peaks at M^+-SCH_3, M^+-H_2O, M^+-HCl and M^+-CH_3. Large peaks at *m/e* 273, 163 and 125 are assigned to ion fragments **VI**, **VII**, **VIII** (Fig. 5), respectively.

Silylation of **V** yielded a compound which showed molecular ion peak at 638 mass units indicating the presence of three trimethylsilyl groups in the molecule. These data establish the bicyclic imidazolidone structure **V** for the crystalline product of the reaction of N-demethylclindamycin and formaldehyde at elevated pH.

As mentioned earlier, compounds A and B, produced when N-demethylclindamycin was added to fermentations of *S. lincolnensis*, were found to have tlc mobilities identical to those of N-demethyl-N-hydroxymethylclindamycin (**IV**) and the bicyclic derivative (**V**), respectively. There is little doubt that compound A is N-demethyl-N-hydroxymethylclindamycin and that this material is produced by microbial hydroxymethylation. Compound B on the other hand, identical to the bicyclic imidazolidone **V**, probably is formed from compound A by dehydration under the fermentation conditions.

The biological properties of N-demethyl-N-hydroxymethylclindamycin and imidazolidone **V** will be reported in a subsequent communication.

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