NOTES

TRANSFORMATIONS OF ANTIBIOTICS
1. MICROBIAL TRANSFORMATION OF ANTIMYCIN A

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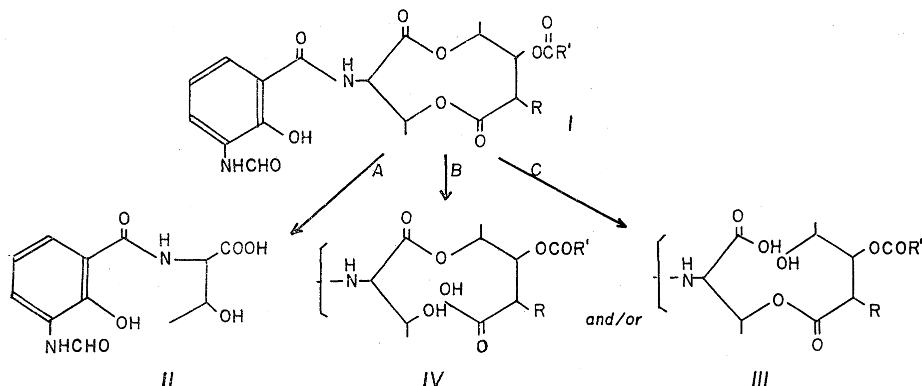
A large number of microorganisms, mainly aspergilli and streptomycetes were tested for their ability to transform antimycin A. Of the few aspergilli that brought about some change in the antibiotic, *Aspergillus ochraceus* (AY F-219) was found to be the most effective and was used for the present study.

Vegetative growth or spores of *A. ochraceus* transformed antimycin A into a more polar product (TLC on silica gel F-254 plates with a solvent system of 20 % ethanol in benzene): the organism was grown on a medium containing 2 % "cerelose" (Corn Products, N. Y.), 2 % "edamine" (Sheffield) and 0.5 % corn steep liquor for 24 hours; antimycin A (0.5 mg/ml) was added and the incubation was continued for 48 to 72 hours at 28°C on a rotary shaker. Alternatively, mycelium from 24 hours growth of the organism was washed with sterile water and resuspended in the original volume of 0.05 M phosphate, pH 7.5~8.0. Antimycin A was added and the mixture incubated for 48~72 hours. Spores were obtained by growing the organisms on moist barley[1]. Maximum yield of transformation product was obtained when antimycin A (0.5~1.0 mg/ml) was incubated with a suspension of spores in 0.02 M phosphate, pH 8.0 at 28°C for 72 hours. Uninoculated controls wherein antimycin A suspensions in buffer or medium were incubated under the above experimental conditions showed no significant degradation of antimycin A. At the end of the incubation period, the reaction mixture was adjusted to pH 5.0; mixed with celite and filtered. The transformation product was extracted from the filtrate. Untransformed antimycin A was recovered from the filter cake by extraction with acetone and determined by the spectrophotofluorometric procedure[2].

In a typical experiment, 4 g of antimycin A dissolved in 40 ml of acetone was added to 4 liters of a spore suspension of *A. ochraceus* (spores, 1×10^9/ml) in 0.02 M phosphate pH 8.0, containing 4 g glucose. Five hundred ml of the suspension were transferred to each 2-liter Erlenmeyer flask and the flasks incubated on a rotary shaker for 72 hours at 28°C. Celite (40 g) was added to the mixture; the pH was adjusted to 5.0 with 1 N HCl and the mixture was filtered. The filtrate was extracted twice with 1/2 volume of CH_2Cl_2. Removal of the solvent gave 956 mg of residue which was chromatographed on silica gel. Elution of the column with carbon tetrachloride-ethylacetate (1:1) yielded 438 mg of a very polar product.



In order to further purify this material, 200 mg of it was acetylated with acetic anhydride in pyridine. After usual work up, the resulting oily product showed a broad band at 3500 cm^{-1} indicating a carboxylic group in the molecule. It was therefore treated with an ethereal solution of diazomethane and the resulting brown oily methyl ester was chromatographed over silica gel. Elution with 30 % ethyl acetate in benzene yielded 140 mg of a clear oil showing the following characteristic peaks in the n.m.r. spectrum in $CDCl_3$: δ 9.4(s)-1H(CHO), 3.7(s)-3H($COOCH_3$), 2.18(s)-6H ($OCOCH_3$), 2.1(s)-3H($OCOCH_3$) and 1.7~0.8(m) (CH_2 and CH_3).

The singlet of the aldehydic proton of the formamido group at δ 9.4 indicates substitution of -NH- during acetylation which accounts for one of the three acetyl peaks. The other two acetyl peaks must then be due to phenolic acetate and from one generated during the microbial transformation. The singlet at δ 3.7 accounts for the carbomethoxy-methyl obtained by diazomethane reaction on the product indicating that the original product was acidic. The acid can be produced by one of the three pathways A, B or C, as shown in the figure.

If the cleavage occurred as in A, the product will be blastamycic acid which would show similar n.m.r. peaks as described above, except for the methylene and methyl peaks, whereas product(s) obtained via pathways B and/or C would give rise to all the characteristic peaks shown in the n.m.r. spectrum of the product after acetylation and esterification. In another experiment, the original degradation product was treated with diazomethane and purified by chromatography over silica gel using 40 % ethyl acetate as eluant. The clear oil showed the following peaks in the n.m.r. spectrum in $CDCl_3$: δ 8.4(d) 1H(CHO), 7.05~7.4(m) 3H (Aromatic), 3.925(s) and 3.9(s)-3H(OCH_3), 3.6(s) and 3.7(s)-3H(-$COOCH_3$). The presence of two methoxyl peaks and two carbomethoxy peaks clearly indicated that the cleavage of the dilactone took place by both the pathways B and C, resulting in two acids showing different chemical shifts for their corresponding methoxyl and carbomethoxy groups. These esters are apparently inseparable by ordinary chromatographic means. The above mixture upon acetylation yielded a diacetate showing in its n.m.r. spectrum a singlet with a shoulder at δ 2.175 for six protons and in which the aldehydic proton collapsed to a singlet indicating further substitution of the formamido NH by acetate. The other peaks (methoxyl and carbomethoxy) remained unaltered. From the above data it may be concluded that the product of antimycin A transformation by *A. ochraceus* is a mixture of two acids **III** and **IV** formed by cleavage of the molecule by the two pathways B and C. This indicates the presence of a lactonase in the organism.

The transformation product had no antibacterial activity. When tested against *Saccharomyces cerevisiae* Y-30 by the cylinder plate method of LOCKWOOD *et al.*[3] as, modified by MURPHY & DERSE (Wisconsin Alumni Research Foundation, personal communication) it showed about 2 % of the activity shown by antimycin A. Compared to antimycin A the product showed a weak effect on respiration of *S. cerevisiae* and *Candida albicans*.

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