NOTES

TRANSFORMATIONS OF ANTIBIOTICS

II. TRANSFORMATION OF ANTIMYCIN A BY HOG KIDNEY ACYLASE

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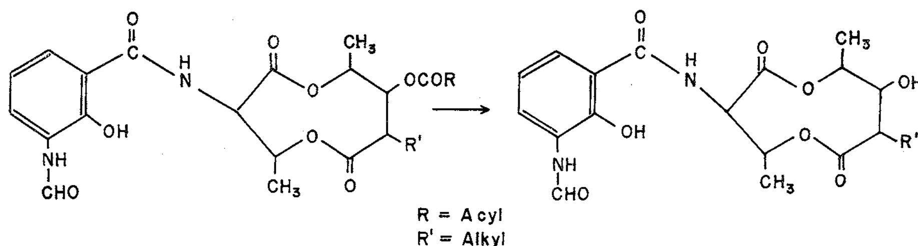
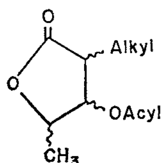
In an earlier communication[1] we reported cleavage of the lactone ring of antimycin A by *Aspergillus ochraceus*. We have observed this reaction with a few other fungi and streptomycetes. With the idea of hydrolysing the ester or the amide bond in antimycin A we studied the action of the following hydrolytic enzymes: trypsin, chymotrypsin, pancreatin, peptidase (from hog intestine), carboxypeptidase A, carboxypeptidase B, wheat germ and pancreatic lipase (from Sigma Chemical Co.); hog kidney acylase (Schwartz/Mann) and a crude preparation of *Escherichia coli* penicillin acylase[2]. Under the various experimental conditions tried, none of the enzymes except hog kidney acylase showed any effect on antimycin A. In this communication we report on the deacylation of antimycin A by hog kidney acylase.



Hog kidney acylase (500 mg) was dissolved in 1 liter of 0.05 M phosphate, pH 8.0. To this solution, 1 g of antimycin A (dissolved in 40 ml of acetone) was added. The mixture was distributed into two 2-liter Erlenmeyer flasks and the flasks incubated on a rotary shaker at 37°C. After 17-hour incubation, 10 g of Celite was added and the mixture filtered. Most of the unreacted antimycin was removed in the filter cake. The transformation product was recovered from the filtrate by extraction with dichloromethane. Evaporation of the solvent gave 196 mg of a residue which, on thin-layer chromatography (t.l.c.) on Merck silica gel F-254 plates, showed small amounts of residual antimycin A and a more polar product. The product was purified by preparative silica gel t.l.c., using 20 % ethanol in benzene for development. The slightly brown material obtained was submitted to chromatography on Sephadex LH-20 in methanol. The fractions containing the product were pooled and evaporated to dryness. The dry material was chromatographed on silica gel (Merck) and the product eluted with 30 % ethyl acetate in benzene. Evaporation of the solvent yielded 44 mg of pure crystalline product. The product showed an ultraviolet spectrum (λ_{max} 226 and 319 nm) and fluorescence response[3] similar to that shown by antimycin A.

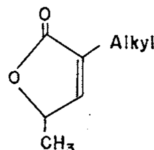
The product was characterized as deacyl antimycin A on the basis of n.m.r. spectroscopy and pyrolysis-gas liquid chromatography (g.l.c.). The n.m.r. spectrum of this product showed the presence of an N-formyl proton at δ 8.2 (d), the aromatic protons δ 6.85~7.6 (m) and the methyl and methylene protons between δ 0.9 and 1.5. This indicated that the basic structure of antimycin was retained in the transformation product. Since this product was more polar than the parent compound and it was not acidic, it seemed that the isovaleryl and butyryl groups

(I) 2-Alkyl-3-acyloxy-4-hydroxy pentanoic acid-γ-lactone



	Alkyl	
A_1	Hexyl	isovaleryl
A_2	Hexyl	butyryl
A_3	Butyl	isovaleryl
A_4	Butyl	butyryl

(II) 2-Alkyl-4-hydroxy-2-pantenoic acid-γ-lactone



A_1	Hexyl
A_2	Hexyl
A_3	Butyl
A_4	Butyl

were hydrolyzed by the enzyme.

In the n.m.r. spectrum of antimycin A, the butyryl group shares a terminal methyl group with two main alkyl groups, hexyl and butyl. In a complex n.m.r. spectrum like the one of antimycin A the absence of butyryl group after enzymatic hydrolysis goes unnoticed. On the other hand, the isopropyl system of the isovaleryl group has a typical doublet representing six methyl protons. The disappearance of this relatively strong n.m.r. signal in the transformation product was observed. The product was acetylated with acetic anhydride and pyridine. Usual workup yielded an oily acetate. The n.m.r. spectrum of this acetate showed three acetyl methyls at δ 2.1 (2-OAc) and δ 2.3 (1-OAc) and the formyl proton now appeared as a singlet at δ 9.4, indicating substitution on the -NH of NHCHO. Of the three acetyls, one being designated for N-acetyl, the other for phenolic acetyl, the third one should be for the hydroxy function liberated during the acylase reaction. Since there was no acid produced in this reaction, the hydroxy group in it must be the one formed from hydrolysis of the acyl group of antimycin A.

Antimycin A, under similar acetylation conditions, produced a diacetate, the acetate methyl appearing at δ 2.1 and 2.3 in the n.m.r. spectrum, indicating facile acetylation of the formamido group in the molecule.

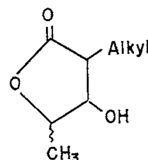
The structure of the acylase reaction product as deacyl antimycin A was further substantiated by the following g.l.c. data.

Pyrolysis-Gas Liquid Chromatography

Pyrolysis of antimycin A leads to only two degradation products which are volatile enough to be chromatographed[5].

Product I is actually a mixture of four isomers which under the g.l.c. conditions described in Table 1 gave rise to two unresolved pairs of peaks (N_i and O_i series in [4]) of about equal intensity. Improved g.l.c. conditions, using a long 12 ft (365 cm) selective column (Table 2), allowed the separation of the four isomers for each alkyl acyl combination. In product II the possibility of isomers no longer exists and thus both g.l.c. conditions show only the two peaks for the 2-butyl and 2-hexyl-4-hydroxy-2-pentenoic acid-γ-lactones.

If antimycin A, by incubation with kidney acylase, has lost its acyl functions none of the 2-alkyl-3-acyloxy-4-hydroxy-pentenoic acid-γ-lactones should be observed in the g.l.c. of its pyrolysate. On the other hand, the two possible 2-alkyl-4-hydroxy-2-pentenoic acid-γ-lactones should be present. If the transformation product from antimycin A has a hydroxyl instead of an acyloxy group, a new pair of peaks due to the 2-alkyl-3, 4-dihydroxy-pentanoic acid-γ-lactone (III).



(III)

should be observed, provided that this compound is thermally stable and does not completely go to the 2-pentenoic acid γ-lactone (II). The 2-alkyl-3,4-dihyroxy pentanoic acid-γ-lactones should also be able to form trimethylsilyl ethers. The pyrolysate of the transformation product from antimycin A did indeed show the above-mentioned characteristics: complete absence of all the possible 2-alkyl-3-acyloxy-4-hydroxy-pentanoic acid-γ-lactones (I); presence of the two 2-alkyl-4-hydroxy-2-pentenoic acid-γ-lactones (II); presence of new peaks due to 2-alkyl-3,4-dihydroxy-penta-

Table 1. Pyrolysis-gas liquid chromatography

Pyrolysate	Peak $M_{3,4}$* 2.65 min.	Peak $M_{1,2}$* 4.75 min.	Peak N_3* 9.65 min.	Peak O_3* 11.0 min.	Peak N_1*	Peak O_1*	New peaks
Pure antimycin A_3 as standard	+	−	+	+	−	−	
Pure antimycin A_3 trimethylsilylated	+	−	+	+	−	−	
Complex antimycin A	+	+	+	+	+	++	
Complex antimycin A trimethylsilylated	+	+	+	+	+	+	
Transformation product	+	+	−	−	−	−	3.85, 4.5, 7.5 and 8.2 min.
Transformation product trimethylsilylated	+	+	−	−	−	−	5.4, 5.8, 10.3, 10.6 and 11.0 min.

Column, 400 cm, 1.2 % SE 30 on Gaschrom Q, 80~100 mesh ; column temperature, 200°C.
* The numbering of the peaks is the same as in reference 5.

Table 2. Pyrolysis-gas liquid chromatography

Pyrolysate	Peak $M_{3,4}$* 1.75 min.	Peak $M_{1,2}$* 2.60 min.	Peaks N_3* 3.90 & 4.20 min.	Peaks O_3* 5.20 & 5.50 min.	Peaks N_1*	Peaks O_1*	New peaks
Pure antimycin A_3 standard	+	−	+	+	−	−	
Pure antimycin A_3 trimethylsilylated	+	−	+	+	−	−	
Antimycin A complex	+	+	+	+	+	+	
Antimycin A complex trimethylsilylated	+	+	+	+	+	+	
Transformation product	+	+	−	−	−	−	8.9, 11.8 min.
Transformation product trimethylsilylated	+	+	−	−	−	−	2.1, 3.2 min.

Column 400 cm, 2.5 % mixture of 3 parts DEGS and 1 part XE 60 on Gaschrom Q, 80~100 mesh ; column temperature, 190°C. * The numbering of the peaks is the same in reference 5.

noic acid-γ-lactones (**III**), from which trimethylsilyl ethers can be formed.

These observations are presented in Tables 1 and 2.

The deacyl derivative of antimycin A showed no antibacterial activity. When tested against *Saccharomyces cerevisiae* Y-30 by the cylinder plate method of LOCKWOOD *et al.*[4] as modified by MURPHY and DERSE (Wisconsin Alumni Research Foundation, personal communication), it showed about 10~15 % 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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