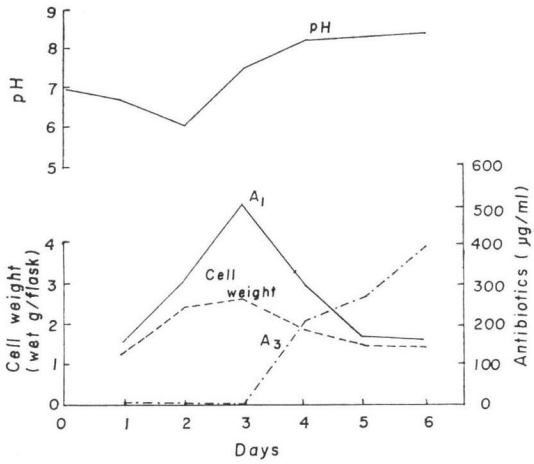
THE ENZYMATIC INTERCONVERSION BETWEEN MIDECAMYCIN A_1 AND A_3

Sir:

Midecamycins (SF-837) are a series of 16-membered macrolide antibiotics, produced by *Streptomyces mycarofaciens*[1,2]. These antibiotics are classified according to the differences of the structures at the 9-position of the lactone ring and of the acyl groups in 4-position of the mycarose moiety[3,4,5]. The structure of midecamycins A_1 and A_3 are shown in Fig. 1. In the course of studies on the biosynthesis of midecamycins, we found that midecamycin A_1 and A_3 were interconvertible by utilization of washed cells of *Streptomyces mycarofaciens* No. 510-19, a high producing strain. SUZUKI *et al* reported the interconversion involving leucomycin A_3, carbomycin A, carbomycin B and maridomycin II by *Streptomyces hygroscopicus*[6]. We report here, the interconversion between midecamycin A_1 and A_3, by the enzyme system extracted from *Streptomyces mycarofaciens* No. 510-19. The lyophilized cells of *Streptomyces mycarofaciens* No. 510-19 were inoculated in a test tube (2× 20 cm), containing 10 ml of the medium which consisted of 0.5% glucose, 1.0% Polypepton, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.3% NaCl, pH 6.8. The inoculum was incubated for 20 hours, at 28°C on a tube shaker. The above inoculum (0.4 ml) was transferred to 250-ml Erlenmeyer flasks containing 30 ml of the medium which consisted of 1.0% yeast extract, 0.5% Polypepton, 0.5% meat extract, 0.5% NaCl and 0.3% $CaCO_3$, pH 6.8. Flasks were shaken on a rotary shaker at 23°C. The time course of the fermentation is shown in Fig. 2. The cells of three flasks at each day were harvested by centrifugation and washed twice with 20 ml of water, and suspended with 10 ml of 20 mM phosphate buffer, pH 6.8. The suspension was disrupted by Polytron (Kinematica, Switzerland), centrifuged at 20,000 *g* for 20 minutes and the supernatant (crude extract) retained. All procedures were performed at 4°C.

Fig. 2. Time course of fermentation (*Streptomyces mycarofaciens* No. 510-19)



Interconversion activity between midecamycin A_1 and A_3 was found in the crude extract, as shown in Table 1. The activity from A_3 to A_1, required NADPH rather than NADH, and the activity from A_1 to A_3, required NADP which was better than NAD. The reaction products were identical with the authentic samples by the UV spectrum, bioautography and nmr data. As shown in Fig. 3, both activities showed very similar patterns and the highest activities were found in the crude extract from the 3-day culture. The activities in the crude extract were not dialyzable and were heat labile. These results suggested the

Fig. 1. Structure of midecamycins A_1 and A_3

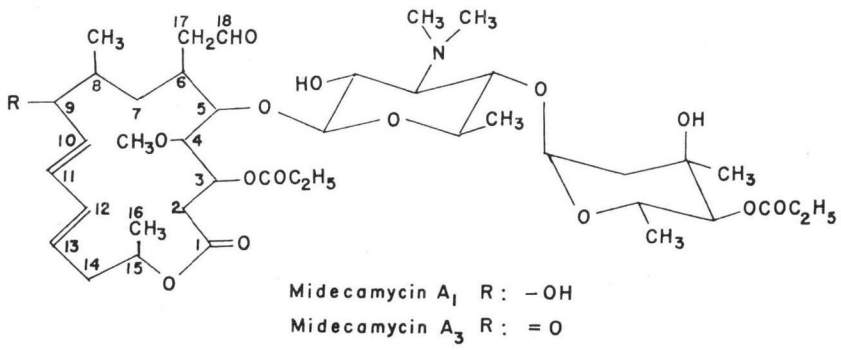


Table 1. The activity of the interconversion.

(1) Midecamycin A_3 to A_1

	Activity (A_1 formed, μg)
Complete mixture (1)	32.3
-NADPH+NADH (250 μg)	11.7
-NADPH	5.1

(2) Midecamycin A_1 to A_3

	Activity (A_3 formed, μg)
Complete mixture (2)	68.4
-NADP+NAD (250 μg)	17.4
-NADP	13.8

Complete mixture (1): midecamycin A_3 100 μg; NADPH 250 μg; the crude extract 200 μl; 50 mM MacIlvaine buffer (pH 6.0); total volume 600 μl.

Complete mixture (2): midecamycin A_1 100 μg; NADP 250 μg; the crude extract 200 μl; 50 mM phosphate buffer (pH 7.5); total volume 600 μl.

The reaction mixture were incubated for 60 minutes at 37°C, stopped with addition of ethylacetate (2.0 ml) and extracted. The organic phase (1.0 ml) were evaporated to dryness, and dissolved with 3.0 ml of methanol.

The amount of midecamycins A_1 and A_3 was determined with the optical density at 230 nm and 280 nm, respectively.

involvement of enzymes in this reaction. Therefore, we tried the purification of enzymes which catalyse both reactions. The activities were fractionated with 30~60% solution of $(NH_4)_2SO_4$, and this fraction was chromatographed on a Sephadex G-100 column. As shown in Fig. 4, both activities were found in the same fractions. The activities were not separated by DEAE-Sephadex A-25 column chromatography. By heating of the enzymes (obtained the Sephadex G-100 column chromatography) at 50°C, for 10 minutes, about 75% of both activities were lost. The molecular weight of the enzymes estimated to be approximately 40,000 by the Sephadex G-100 column chromatography method. The optimum pH for the reduction of the lactone ring of midecamycin A_3 was about 6.0 and 7.5 for the oxidation of the lactone ring of medecamycin A_1.

From these results, we speculated that this oxidoreduction was catalysed by the same enzyme, as shown in Fig. 5. The enzyme (or

Fig. 3. The interconversion activity of the crude extract.

The formation of midecamycin A_1 or A_3 were assayed as shown in Table 1.

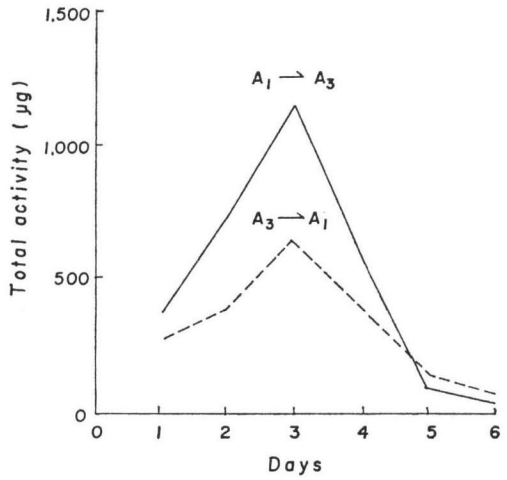


Fig. 4. Sephadex G-100 chromatography of the crude enzymes.

Buffer: 20 mM phosphate buffer, pH 6.8
Column size: 19×490 mm
Sample: 2.0 ml

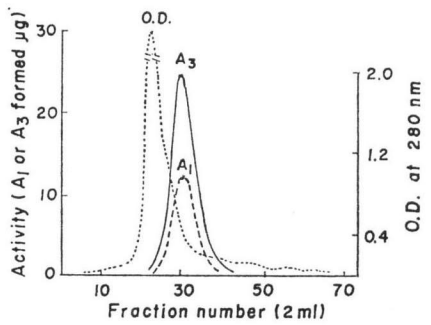
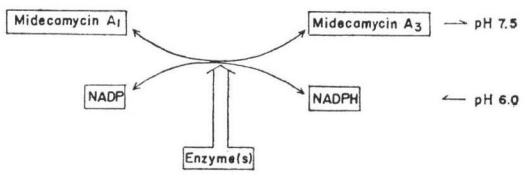


Fig. 5. Summary of the enzymatic reaction



enzymes) did not catalyse the reduction of the aldehyde group in the 18-position of the lactone ring. We believe that the enzyme (or enzymes) is related to the biosynthesis of midecamycins, because such activity was not detected in some

other *Streptomyces* which do not produce macrolide antibiotics.

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