ON THE SYNERGISM OF SF-973 B AND C

Sir:

An interesting synergistic phenomenon in the appearance of the antibiotic activity by the combination of two inactive substances has been described in two cases in the past, that is MSD-235 S and L[1], and geminimycins A and B[2].

In this communication we wish to report another example of this type of synergim demonstrated by SF-973 B and C, and also describe the mechanism of the appearance of the antibiotic activity in terms of enzymatic conversion of SF-973 C (dihydroabikoviromycin) to SF-973 A (abikoviromycin) in the presence of SF-973 B.

Streptomyces olivaceus SF-973 was cultured at 28°C for 48 hours in the medium containing 2.5 % glucose, 2.0 % wheat embryo, 0.5 % soluble vegetable protein and 0.25 % NaCl (pH 7.0). The bioautogram of the broth filtrate thus obtained showed the presence of a single bioactive compound, which was named SF-973 A and purified as shown in Chart 1. It is a very unstable compound, decomposing to reddish brown compounds upon standing at room temperature. It shows UV maxima at 236 mμ ($E^{1\%}_{1cm}$ 620) and 338 mμ ($E^{1\%}_{1cm}$ 720) in acidic water, and in alkaline water at 246 mμ ($E^{1\%}_{1cm}$ 540) and 290 mμ ($E^{1\%}_{1cm}$ 480). The substance SF-973 A shows a broad antibacterial spectrum against gram-positive and gram-negative bacteria, and mycobacteria. Its extreme lability, the position of UV maximum and the spectral pattern of SF-973 A resembled those reported for abikoviromycin[3] (latumcidin[4,5]), and the identity of both compounds was established by direct comparison of behavior of TLC, and of UV, IR and NMR spectra[6,7].

In the course of the isolation procedure for SF-973 A, it was found that the ethyl acetate extraction of the culture filtrate at pH 9 resulted in a considerable loss of bioactivity, but recombination of the aqueous layer and the solvent extract recovered most of the antibiotic activity observed in the original broth, suggesting the synergism of several components. Therefore, the nature of these components was persued by utilizing as a bioassay the synergistic phenomenon on the agar plate of *Klebsiella pneumoniae*.

At the early stage of the investigation, SF-973 A was excluded as a candidate of synergistic components, since it exhibited no synergism with either the aqueous layer or the solvent extract. However, when the aqueous layer was combined with the solvent extract free from SF-973 A, a definite bioactivity appeared in the solution. These results clearly indicate that at least two components exist, one in the aqueous layer and the other in the solvent extract. They were isolated as follows:

Chart I. Isolation and purification of SF-973 A, B and C.

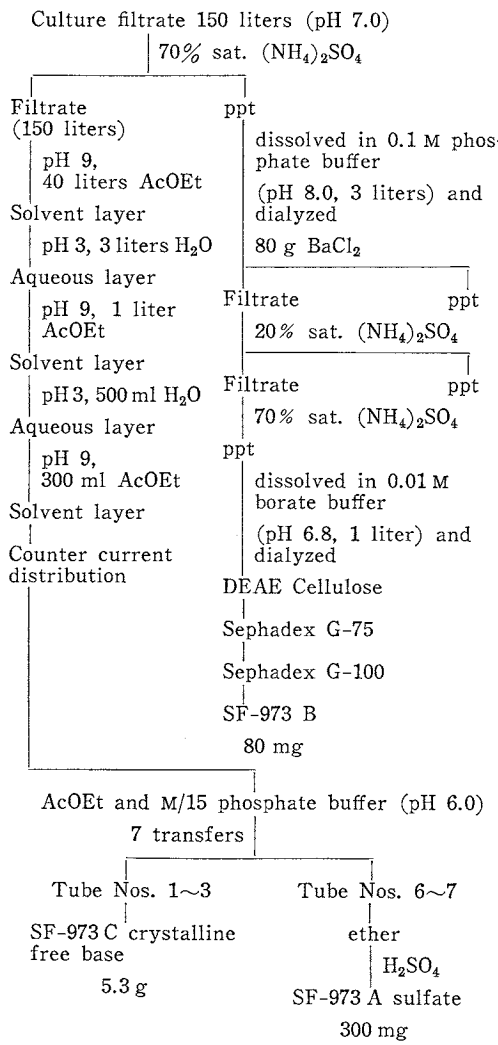
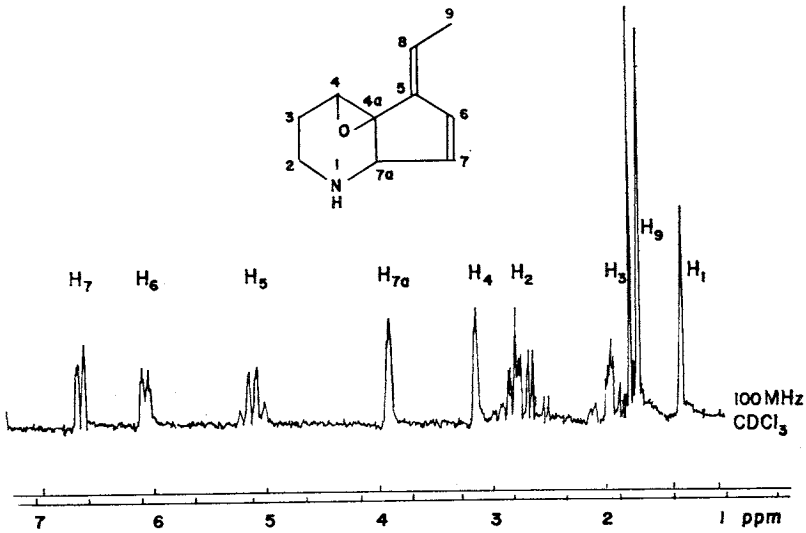


Fig. 1. NMR spectrum of SF-973 C.

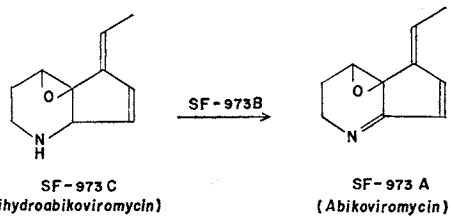


Seventy percent saturation of the aqueous layer with ammonium sulfate caused a precipitation. The precipitate was treated by the procedures used for the purification of proteins (Chart 1), and a substance of protein nature was obtained and named SF-973 B. Approximate molecular weight estimated by the gel filtration was 36,000.

The ethyl acetate extract was further separated as shown in Chart 1, and in addition to SF-973 A a new crystalline compound designated SF-973 C was obtained. It shows m.p. 57~59°C (free base), elementary analysis; C 74.25, H 8.06, N 8.81 %, MW; 163 (mass spectrometry), molecular formula; $C_{10}H_{13}NO$, $[\alpha]_D^{22}$ +276° (*c* 1, MeOH), pKa 7.7 (50 % EtOH), and UV maximum in MeOH at 244 mμ ($E_{1cm}^{1\%}$ 850). It is easily crystallized as the hydrochloride that shows m.p. 130~132°C and is soluble in water. The NMR spectrum of the free base (Fig. 1) indicated the presence of an ethylidene group at 5.12 ppm (1H, quartet) and 1.86 ppm (3H, doublet), and a *cis*-disubstituted ethylene group at 6.06 ppm (1H, doublet of quartet) and 6.65 ppm (1H, doublet of doublet).

These results together with the UV band at 244 mμ suggested the following conjugated chromophore, CH_3-CH=C-C=C- as a partial structure. At this stage it was noted that the NMR spectrum, molecular weight and formula were close to those of SF-973 A (*i. e.* abikoviromycin, MW, 161, formula, $C_{10}H_{11}NO$), and therefore dihydroabikoviromycin was proposed as the most propable structure of SF-973 C. This postulation was fully substanciated by direct comparison in NMR, IR, $[\alpha]_D$ and TLC of SF-973 C with the authentic dihydroabikoviromycin which was obtained by hydrogenation of abikoviromycin with $NaBH_4$ [6]. To the best of our knowledge, this is the first instance where dihydroabikoviromycin was isolated directly from a culture broth.

Scheme 1. Conversion of SF-973 C to SF-973 A in the presence of SF-973 B.



Each of the SF-973 B and C alone showed no antibacterial activity, but, when both substances were combined, there appeared a strong bioactivity. In a typical experiment, 100 mg of SF-973 C hydrochloride in 30 ml of 0.1 M phosphate buffer (pH 7.0) was incubated with 20 mg of SF-973 B at 30°C for 30 minutes, and the incubated solution was extracted with ethyl acetate at pH 9.0.

Examination of the bioactive extract by silica gel TLC (AcOEt-MeOH, 10 : 1) revealed the complete disappearance of SF-973 C (Rf 0.2) and the formation of a new bioactive compound, whose Rf value 0.5 was identical with that of SF-973 A. Indeed the newly formed compound obtained as a crystalline sulfate (30 mg) was identified as abikoviromycin sulfate by comparison of the UV, IR and NMR spectra.

Based on the observations described above, the synergism of SF-973 B and C could be rationalized, if SF-973 B is an enzyme that catalizes the dehydrogenation of SF-973 C (dihydroabikoviromycin) to SF-973 A (abikoviromycin) as shown in Scheme 1.

The enzymatic properties of SF-973 B were further studied; it showed the maximum activity at pH 6.5~8.5 with optimal temperature of 45~55°C. No activity loss was observed when SF-973 B was kept in buffer solution over a pH range of 6.5~8.5 at 35°C for 20 hours. Enzymatic reaction did not occur when the solution was free from oxygen. Further properties of SF-973 B will be the subject of a forthcoming paper.

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