Communication to the editors

ISOLATION OF RIFAMYCIN SV FROM A MUTANT *STREPTOMYCES MEDITERRANEI* STRAIN

Sir:

Rifamycin SV (Fig. 1) is a semisynthetic antibiotic obtained[1] by chemical modification of rifamycin B, a product of *Streptomyces mediterranei* fermentation[2,3]. Studies on the biogenesis of rifamycin B indicated[4,5] that rifamycin SV in a precursor of rifamycin B in *S. mediterranei* fermentations, since ^{14}C rifamycin SV is converted in high yields into labelled rifamycin B both by growing cultures or by washed mycelium of this microorganism.

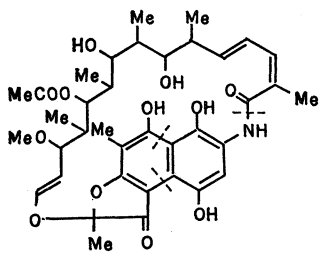
Therefore it appeared possible to obtain a blocked mutant of *S. mediterranei* strain which directly produces in submerged cultures rifamycin SV, or its oxidized analog rifamycin S. A screening program was performed, by treating mycelium suspensions of *S. mediterranei* with N-methyl-N'-nitro-N-nitrosoguanidine and testing the surviving colonies for their ability in inhibiting the growth of *Pseudomonas reptilivora* on Penassay-agar plates at a pH of 7.2. In these conditions rifamycin B is inactive, while concentrations of 50 μg/ml of rifamycin SV can be detected.

One of the strains thus isolated (ATCC 21271) was grown at 28°C in Erlenmeyer flasks in a medium containing (g per liter) peanut meal 25, soybean meal 5, $(NH_4)_2SO_4$ 9.5, $MgSO_4 \cdot 7H_2O$ 8.85, glucose 95, glycerol 40, propylene glycol 5, KH_2PO_4 1, $CaCO_3$ 8.5, sodium diethylbarbiturate 1.7.

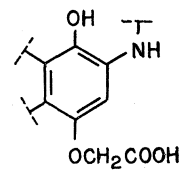
After 120-hour incubation spectrophotometric evaluation gave about 1,800 μg/ml of rifamycins based on absorption at 450 mμ. A semiquantitative determination by thin-layer chromatography indicated that rifamycin SV represented about 75 % of the rifamycins present. Rifamycins B and Y were also evident. For a positive identification the antibiotics were recovered and purified as follows. The broth was treated with 5 g/liter of ascorbic acid in order to maintain rifamycins in their reduced, more acidic form, and filtered at pH 7, acidified to pH 3 and extracted three times with half a volume of ethyl acetate. The extracts were pooled, and the solvent evaporated under reduced pressure. The residue was absorbed onto a chromatographic column of silicagel (Merck) and eluted with acetone. Fractions showing only one spot at Rf 0.90 in thin-layer chromatography (Silicagel G, acetone solvent) were pooled, and the solvent evaporated to a small volume. The product, precipitated by addition of petroleum ether, was identified as rifamycin SV on the basis of its UV and IR spectra, chromatographic behavior in different systems and biological activity. A further confirmation was obtained by oxidizing the product in ethyl acetate solution with MnO_2. Pure rifamycin S was obtained which, after crystallization from methanol, was found identical with an authentic sample[1] in its physico-chemical and biological properties.

The isolation of rifamycin SV from a

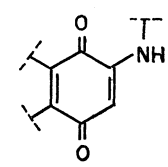
Fig. 1



Rifamycin SV



Rifamycin B



Rifamycin S

mutant *S. mediterranei* strain further confirms the hypothesis that this compound, or its quinonic form rifamycin S, is the precursor of rifamycin B in fermentations, and probably the precursor of rifamycin O isolated by SUGAWARA *et al.*[6] from fermentations of *Streptomyces* strain 4107 A2.

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