ON THE BIOLOGICAL INACTIVITY OF 4,5-DIHYDROXY-*n*-DECANOIC ACID-4-LACTONES

Sir:

In a recent communication we reported on the isolation of diastereomeric 4,5-dihydroxy-*n*-decanoic acid-4-lactones (**1**, **2**) from cultures of *Streptomyces griseus* and suggested that these butyrolactone derivatives were responsible for the autoregulatory effects exerted by the isolated products[1]. However, biological tests with the indicator strain ZIMET 43682[2] performed subsequently by the use of racemic **1** and **2** obtained by synthetic methods[3] proved to be at variance with this proposal and indicated that the biological activity has to be ascribed to some other component of the isolated products. The fact that this component remained undetected in spite of our careful isolation procedures showed that the true autoregulator must have a very high specific activity and occur at extremely low concentrations in the cultures. Further improvements in the cultivation, isolation, and detection procedures were therefore sought and elaborated. One of the major modifications with respect to our former methodology consisted in the suppression of the anthracycline pigment production, a measure taken in order to diminish the loss of the active product due to its comigration with pigments during chromatography. Experiments have revealed that zero time addition of inorganic phosphate ions suppressed the formation of both anthracyclines and lactone **2** by the parental strain *S. griseus* without any noticeable changes in the production of the autoregulator (Fig. 1).

Apart from its practical consequences in the isolation procedures, this observation suggests that, at least for the strains studied, the production of the antibiotic is not directly connected

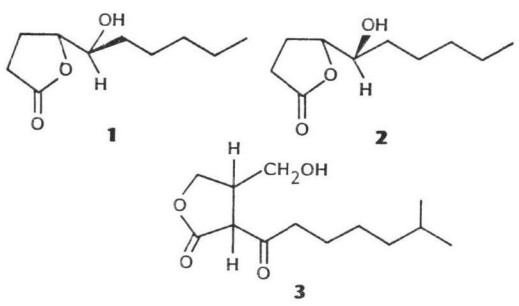
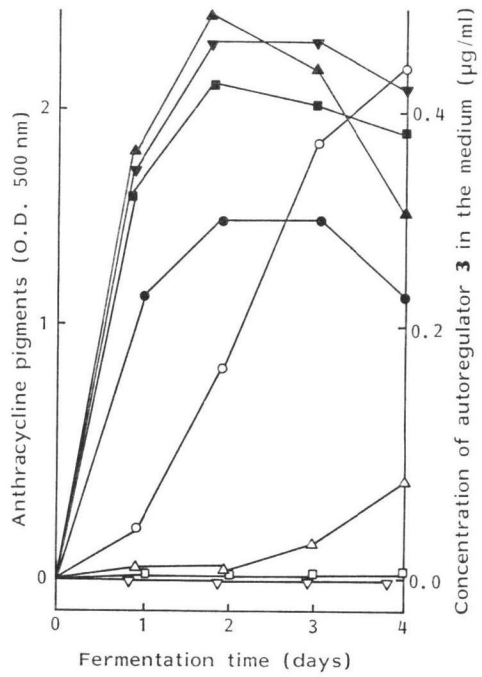


Fig. 1. Production of autoregulator **3** and anthracycline pigments.

Production of autoregulator **3** (A-factor) (shaded symbols) and anthracycline pigments (open symbols) in the course of cultivation of *S. griseus* JA 5142 on complex medium (g/liter: glucose 5, potato starch 30, soya meal 20, NaCl 2, $CaCO_3$ 3, NH_4NO_3 3, pH 6.2; 48-hour inoculum on on the same medium starting with an agar slant culture).

○ No phosphate added. Additions of KH_2PO_4 at zero time (pH 6.2): △ 0.08%; □ 0.16%, ▽ 0.5%. Concentration of autoregulator was determined by bioassay[2] with the culture liquid. The absorbance (O.D. 500 nm) of the $CHCl_3$ extract (1 ml) of 100 ml culture liquid has been taken as a measure for the production of anthracycline pigments.



with the formation of the autoregulator. Another modification of the isolation procedures consisted in the use of different solvent systems at various stages of chromatographic purification (benzene - ether, 1: 1; $CHCl_3$ - methanol, 95: 5; diisopropylether - ethyl acetate, 9: 1; several-fold development in each solvent) instead of the unique solvent system (benzene - ether, 1: 1) used in our previous work. From 500 liters culture liquid of *S. griseus* JA 5142, the modified cultivation and isolation procedures afforded 0.9 mg of the active product. Its Rf value on Silufol sheets (Kavalier, CSSR; 0.6 benzene - ether, 1: 1, four-fold development; 0.8 $CHCl_3$ -

methanol, three-fold development) and EI MS data (Jeol JMS-D100, 75 eV, 100°C, direct inlet: *m/z* 242.1524, M^+ (242.1518 calcd. for $C_{13}H_{22}O_4$), 211.1325 (211.1334 calcd. for $C_{12}H_{19}O_3$; M^+-CH_3O), 171.0670 (171.0657 calcd. for $C_8H_{11}O_4$); 143.0346 (143.0344 calcd. for $C_6H_7O_4$) and biological activity (approx. 5 ng were needed at least in the paper disc assay[2]) proved identical with the respective values reported for A-factor **3**[1,4].

It may be of interest to note that the formation of autoregulators may be accompanied by the production of larger amounts of closely related but entirely inactive molecules like **1** and **2**.

Acknowledgments

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