MICROBIAL DECOMPOSITION OF SALINOMYCIN

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Salinomycin[1] (I) belongs to the group of polyether antibiotics which are widely used as coccidiostats and growth promotors in livestock husbandry[2]. Little information is offered about the compound's behavior in soil. This study examines the microbial enzymatic decomposition of salinomycin by bacteria isolated from soil.

In the past, it was observed that salinomycin is broken down in soil (K. HEIL and W. SAMBETH, unpublished). Two strains of bacteria which are capable of deactivating the compound have now been isolated from soil samples. The strains were identified as *Pseudomonas stutzeri* and *Enterobacter agglomerans* using the API 20E and API 21NE systems (Analytab Products Inc.). Both *P. stutzeri* FH 1796 and *E. agglomerans* FH 1793 are resistant to salinomycin and thus differ from the types mentioned in the literature[3,4].

The decomposition of salinomycin was assessed as follows: Soybean-Casein-Digest-Medium (Merck 5459) (1,000 ml) was inoculated with the *Pseudomonas stutzeri* FH 1796 isolate and incubated under constant stirring for 48 hours at 37°C. The culture was then heated to 80°C for 30 minutes to release the salinomycin degrading enzymes. The pH of the mixture was 7.2. After cooling down to 37°C salinomycin-sodium (10 g) dissolved in methanol (100 ml) was slowly added to the bacterial culture under constant stirring. The resulting mixture was further incubated under constant stirring for 20 hours at 37°C. The subsequent decrease in microbiological activity and the TLC findings showed that the salinomycin had to a large extent decomposed. TLC (solvent system: water - satd EtOAc; staining: vanillin/sulfuric acid) further revealed the occurrence of a new substance at Rf 0.55.

To isolate the product, the reaction mixture was extracted with EtOAc. The extract was purified by column chromatography using silica gel impregnated with phosphate buffer 3%, pH 8.0 and reactivated at 120°C. The eluent was an *n*-hexane - EtOAc gradient. The purified decomposition product of salinomycin (II) crystallized out of *n*-heptane with a yield of 3.5 g: MP 135~136.5°C, $[\alpha]_D^{21} -32°C$ (*c* 1, $CHCl_3$). No

Fig. 1. Electron impact mass spectrum of II.

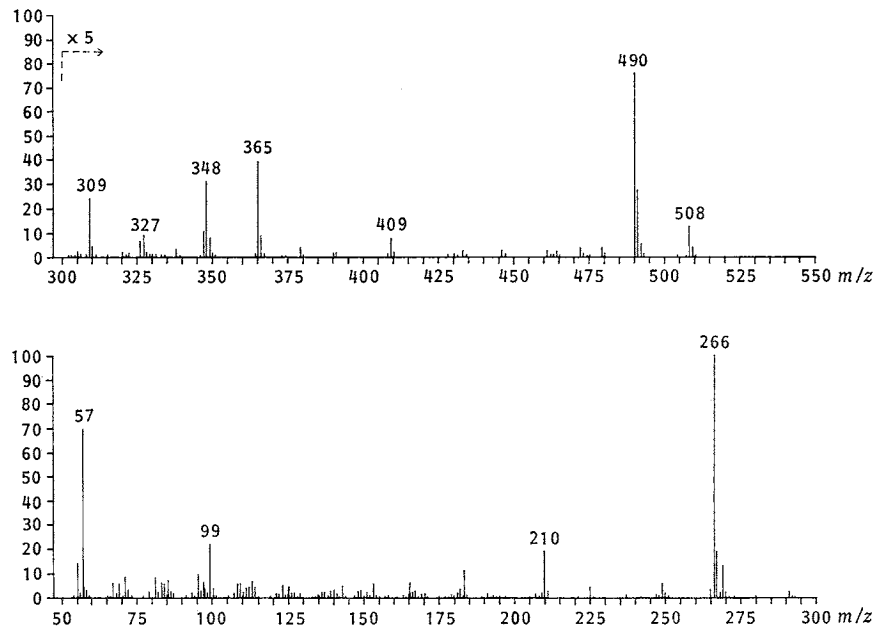
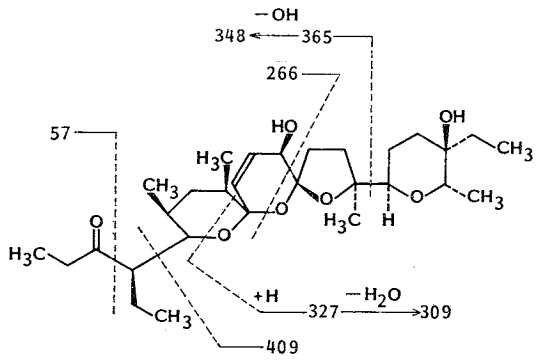


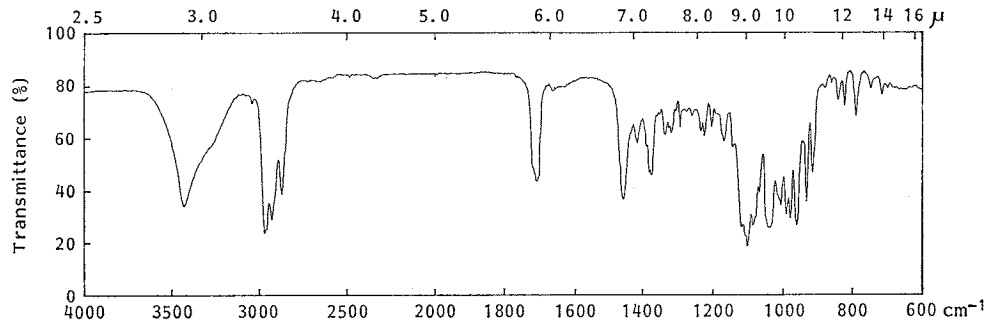
Fig. 2. Rationalization of structurally significant electron impact induced fragmentation reactions of **II** according to high resolution mass measurements. $M^+ = m/z$ 508, $C_{29}H_{48}O_7$.

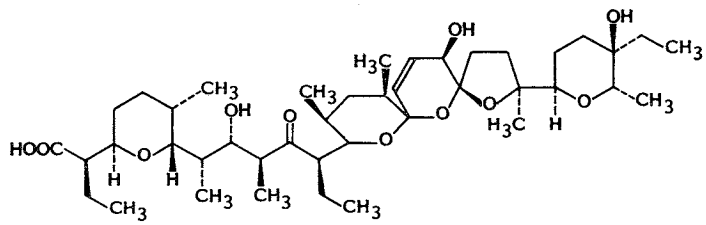


mentionable amounts of other decomposition products were detected in the EtOAc extract by vanillin/sulfuric acid staining.

The structure (II) of the decomposition product was derived from the ^1H NMR and mass spectra. The NMR spectrum measured in deuteriochloroform solution at 270 MHz is rather complex and strongly dependent on the water content of the solution, obviously because of slow equilibria between different conformeric forms of the molecule. Yet the presence of seven *C*-methyl groups (0.7~1.3 ppm), four methine groups attached to oxygen (3.4~4.0 ppm), and two olefinic protons (5.9~6.0 ppm) could be detected. On the other hand, **II** gives a highly characteristic mass spectrum (Fig. 1).

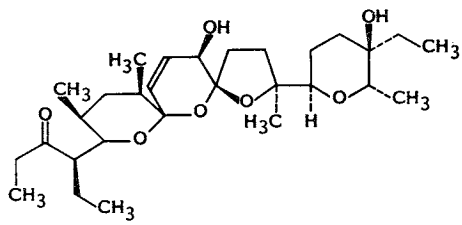
Fig. 3. IR spectrum of **II**.





I

Enzymatic retroaldol cleavage by *Pseudomonas stutzeri*



II

The electron impact induced fragmentation is very similar to salinomycin[5] (I) and indicates the presence of the unchanged C-10 to C-30 part of the molecule, *i.e.* carbon atoms 1 to 9 have been lost (see Fig. 2).

Compound II is a decomposition product of salinomycin produced by enzymatic retroaldol cleavage of the β-hydroxy-ketone group. This type of cleavage is apparently easy, since it can also be observed in the mass spectrometric fragmentation of salinomycin[5].

Compound II is no longer capable of complexing sodium or potassium; no antibiotic activity could therefore be detected. The cleavage product is toxicologically harmless, the oral LD_{50} being in excess of 4 g/kg body-weight in mice.

References

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