AN ANTIBIOTIC SEN-34

Masakuni Ozaki, Yoji Ezure, Takashi Okubo,
Kimio Yamane and Shingo Matsumura*
Research Laboratories, Nippon Shinyaku Co. Ltd.,
Nishioji, Kyoto, Japan

Production, isolation and characterization of antibiotic SEN-34 are described.

In the course of our search for new antibiotics, a hitherto undescribed antibiotic, designated Antibiotic SEN-34, was isolated from the culture broth of a strain of Streptomyces sp. which also produced the known antibiotic, streptonigrin[1]. We now wish to report the production, isolation and characterization of antibiotic SEN-34. Preliminary taxonomic studies demonstrated that the organism producing antibiotic SEN-34 could be readily differentiated from Streptomyces flocculus, Streptomyces bottropensis, Streptomyces fungicidicus and Streptomyces rufochromogenes, which produced streptonigrin.

The fermentation was carried out at 27-30°C for 40 hr in aerated jars with stirring in a liquid medium containing glucose, dry soybean powder, NaCl, $CaCO_3$, and water.

The fermentation broth was filtered and adjusted to pH 4.0. The filtrate was extracted twice with ethyl acetate. The active materials were reextracted into water phase by treating the solvent twice with a buffer solution (pH 8.0). The water layer, after acidification, was extracted again with ethyl acetate. The ethyl acetate extract was washed with a small quantity of water at pH 6.0 and concentrated *in vacuo* to dryness. The residue was dissolved in methanol to be separated into methanol-insoluble and soluble fractions. The methanol-insoluble residue was dissolved in a large volume of chloroform and chromatographed on a column of silica gel using solvent system of chloroform-methanol (80:1). The active residue thereby fractionated was recrystallized from pyridine-ethanol (1:40) to give streptonigrin as dark brown crystals, mp 266°C (decomp.), whose physicochemical data were identical with those of authentic sample isolated by Rao *et al*[1]. The methanol-soluble fraction was passed through a column of silica gel. The column was eluted with chloroform-methanol (9:1) and the solvent was distilled off under reduced pressure. The crystalline residue was recrystallized from chloroform and antibiotic SEN-34 was obtained as orange prisms, mp 161-162°C (Calcd. for $C_7H_7O_5N$: C, 45.41; H, 3.81; N, 7.57; OCH_3, 16.76. Found: C, 45.53; H, 3.76; N, 7.44; OCH_3, 16.68 %), λ_{MeOH}^{max} mμ (ε): 215 (8732), 275 (9309) and 385 (1924), ir (KBr): 3350 (OH), 1735 (CO), 1710 (CO), 1690 (CO) and 1680 cm^{-1} (CO).

Antibiotic SEN-34 was acidic substance and changed abruptly to violet on addition of base. The violet color disappeared immediately, suggesting the decomposition of SEN-34. Antibiotic SEN-34 exhibited a sharp OH absorption band at 3350 cm^{-1}. It gave a negative reaction to ferric chloride test. These facts suggest that there is no indication of the OH---O=C type of hydrogen bond. The structure of antibiotic SEN-34 was deduced from nmr and mass spectral data. In nmr spectrum of antibiotic SEN-34 the signals at 3.20 and 4.03 ppm suggested the presence of NCH_3 and OCH_3 groups, respectively. The mass spectrum of antibiotic SEN-34 exhibited relatively simple fragmentation (Figure 1). As shown in Chart 1, several possible fragmentation pathways were considered as plausible; the one is the step-wise loss of carbon monoxide from the molecular ion at m/e 185, and the others are the cleavage of CH_3-N=C=O molecule and the elimination of -N=CH_2 radical.

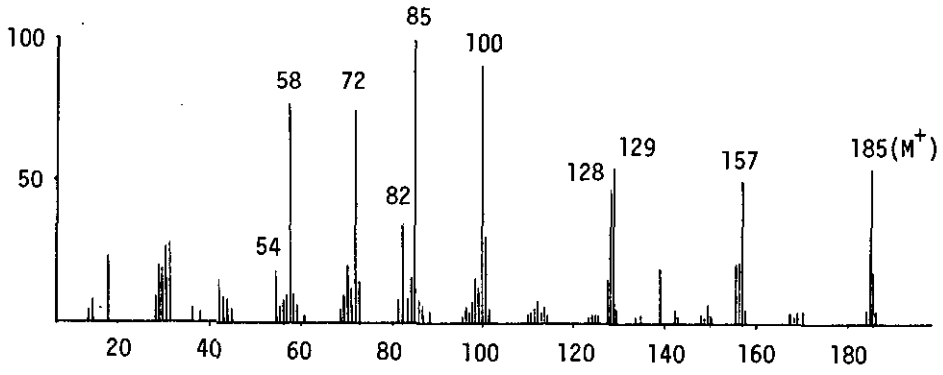
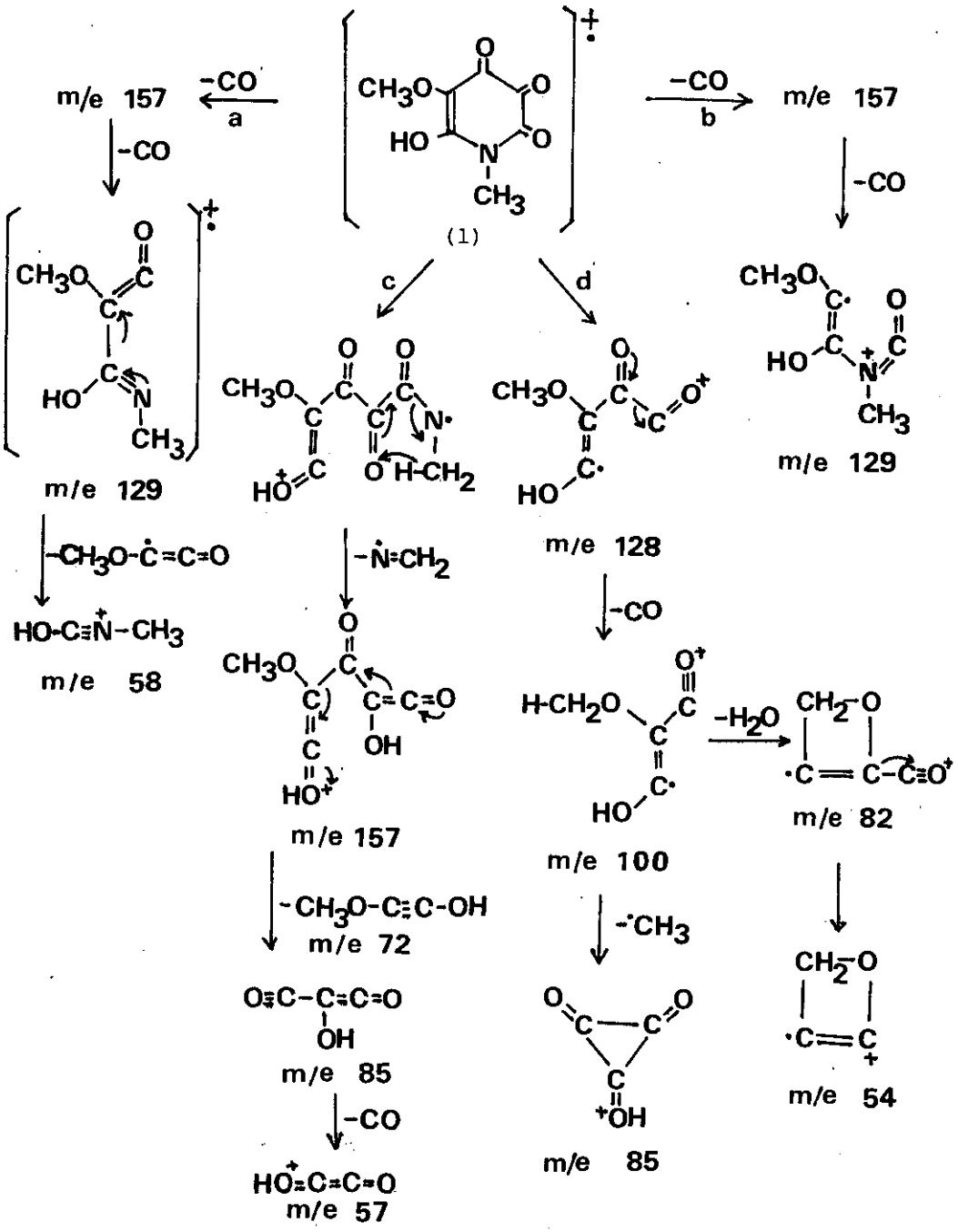


Fig. 1. Mass Spectrum of Antibiotic SEN-34

Chart 1


m/e 157
-CO
a
-CO
b
m/e 157
-CO
(1)
c
d
m/e 129
m/e 129
-CH3O-C≡C=O
HO-C≡N-CH3
m/e 58
-N≡CH2
m/e 157
-CH3O-C≡C-OH
m/e 72
O≡C-C=C=O
OH
m/e 85
-CO
HO≡C=C=O
m/e 57
m/e 128
-CO
m/e 100
-H2O
m/e 82
-·CH3
m/e 85
m/e 54

The fragmentation mechanism was confirmed by the distinct metastable ions (m* 133.0, 105.8 and 89.0) seen in the mass spectrum of antibiotic SEN-34.

These observations described above led us to propose the structure (1), 6-hydroxy-5-methoxy-1-methyl-1,2,3,4-tetrahydropyridine-2,3,4-trione, for antibiotic SEN-34.

Antibiotic SEN-34 exhibited weakly antimicrobial activities against gram-positive and gram-negative bacteria, and was relatively toxic to mice (LD_{50}, ip, 160 mg/kg).

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REFERENCES

1 K. V. Rao and W. P. Cullen, Antibiotics Ann., 1959/60, 950.

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