

### Communications to the editor

#### STRUCTURE OF ANTIBIOTIC DE-3936

Sir:

Antibiotic DE-3936 is the major compound of biologically active substances produced by a strain of *Streptomyces hygroscopicus*. It is active against Gram-positive bacteria, mycobacteria, mycoplasma and protozoa, and it is especially effective in treatment of coccidiosis in poultry.

In this communication, we wish to report the structural elucidation of antibiotic DE-3936 by X-ray crystallographic analysis of the corresponding silver salt.

Antibiotic DE-3936 is a monocarboxylic acid, m.p. 109~114°C; pKa=5.9 (66% acetone);  $[\alpha]_D^{20}$  66.6° (c 1, CHCl<sub>3</sub>);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1730 cm<sup>-1</sup> (C=O);  $\lambda_{\text{max}}$  no absorption above 210 nm.

The molecular formula C<sub>44</sub>H<sub>76</sub>O<sub>14</sub> for DE-3936 was determined on the basis of elemental analysis and its mass spectrum. The mass spectrum of its sodium salt shows *m/e* 850 (M<sup>+</sup>), *m/e* 835 (M-CH<sub>3</sub>), *m/e* 819 (M-OCH<sub>3</sub>), *m/e* 806 (M-CO<sub>2</sub>), and its potassium salt similarly shows *m/e* 866 (M<sup>+</sup>), *m/e* 851 (M-CH<sub>3</sub>), *m/e* 835 (M-OCH<sub>3</sub>), *m/e* 822 (M-CO<sub>2</sub>). These molecular ion peaks are consistent with the molecular formulae C<sub>44</sub>H<sub>76</sub>O<sub>14</sub>Na and C<sub>44</sub>H<sub>76</sub>O<sub>14</sub>K.

The <sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>) shows the presence of four methoxyl groups ( $\delta$  3.3~3.5 ppm) and the <sup>13</sup>C-NMR spectrum (CDCl<sub>3</sub>) indicates the presence of four -O-CH<sub>3</sub> ( $\delta$  56.0, 56.7, 57.3, 60.0 ppm), -COOH ( $\delta$  173.8 ppm), three -O-C-O- ( $\delta$  98.5, 100.0, 106.6 ppm) and ten or more methyl groups in 44 carbons.

However, it is difficult to determine the molecular structure from these data. An X-ray crystallographic analysis was therefore undertaken. The structure of other polyether antibiotics such as grisorixin<sup>1)</sup>, lysocellin<sup>2)</sup> and septamycin<sup>3)</sup> were determined by X-ray analyses using their silver salts or *p*-bromophenacyl esters, therefore the silver salt of DE-3936 was prepared.

The silver salt of DE-3936 was obtained by treating the sodium salt of DE-3936 with AgNO<sub>3</sub>. Recrystallization from methanol-water solution yielded colorless prisms, which

belong to the orthorhombic system with the unit cell dimensions of  $a=15.818$ ,  $b=25.821$  and  $c=12.776$  Å. The space group was determined to be P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> by the systematic absences. The unit cell contains four molecules. Lattice constants and intensities were measured on a PHILIPS four-circle diffractometer with graphite-monochromated CuK $\alpha$  radiation ( $\lambda=1.5418$  Å). The crystal was sealed in a glass capillary since it easily decomposed with loss of solvent of crystallization. The size of the crystal was about 0.35 × 0.25 × 0.20 mm.

The 2955 reflexions in the range  $2\theta < 110^\circ$  were collected using  $\theta-2\theta$  scan technique with  $\theta=4^\circ \text{ min}^{-1}$  scan speed. During the data collection the intensities of the three standard reflexions were decreased due to radiation damage. The amount of the decrease was about 20%. The intensities were normalized using those of the standard reflexions and then corrected for the LORENTZ and polarization factors. The structure factors were converted to the absolute scale by WILSON's method. No absorption correction was applied. The heavy atom position was determined by a PATTERSON synthesis and the other atom positions were found progressively by several cycles of structure factor calculation and FOURIER synthesis. Finally, a difference-FOURIER synthesis revealed the presence of two methanol molecules. Refinement of the positional and thermal parameters was carried out by the block-diagonal least-squares method. The final R value was 0.14. The absolute configuration was determined by the use of anomalous scattering effect of the silver atom for CuK $\alpha$  radiation.

The resulting molecular structure of DE-3936 is shown in Fig. 1. Fig. 2 is the stereoscopic drawing of DE-3936 silver salt which represents the absolute configuration. The conformation is fixed by the hydrogen bond

Fig. 1. Structure of antibiotic DE-3936

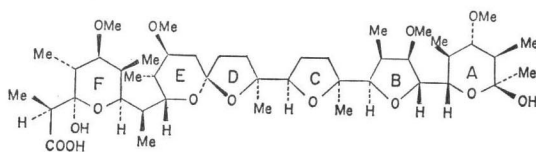
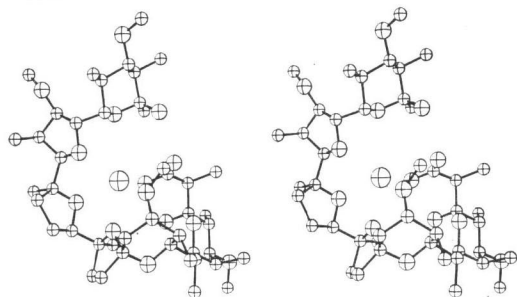


Fig. 2. Stereoscopic drawing of DE-3936 silver salt



(2.69 Å) between the terminal hydroxyl group and one of the carboxylate oxygen atoms and by the interactions between the silver ion and the six oxygen atoms (on B, C, D ring, the carboxyl, and methoxyl in ring E) which lie with distances less than 3.00 Å to the silver ion. The six rings are folded about the silver ion just in the same way as in nigericin<sup>4)</sup> and monensin<sup>5)</sup> etc. The chemical structure is found to be very similar to grisorixin and nigericin, but differs from them in the numbers and positions of methoxyl, methyl and hydroxyl groups.

The results of the studies were presented\* at the 199th Scientific Meeting of J.A.R.A. (September 19, 1975, Tokyo, Japan), where the structure of Ionomycin,\*\* independently elucidated by Dr. ŌTAKE and his associates, was also reported and proved to be identical with that of DE-3936.

\* The announced absolute configuration of DE-3936 was clarified to be reversed after more studies.

\*\* ŌMURA, S.; S. MACHIDA, J. SAWADA, I. TANAKA and N. ŌTAKE: "New antibiotic Ionomycin" Annual Meeting of the Agricultural Chemical Society of Japan (July 23~25, 1975), meeting abstract: page 84

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