

OKILACTOMYCIN, A NOVEL ANTIBIOTIC PRODUCED  
BY A *STREPTOMYCES* SPECIES

## II. STRUCTURE DETERMINATION

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Structure of a novel antibiotic, okilactomycin, was determined by a combination of spectroscopic and X-ray crystallographic studies. Okilactomycin has a unique structure containing 13-membered ring cyclized by carbon-carbon bond.

Okilactomycin isolated from a culture broth of *Streptomyces griseoflavus* subsp. *zamamiensis* subsp. nov. inhibited the growth of tumor cells and that of Gram-positive bacteria.<sup>1)</sup> We will report herein the structure determination of okilactomycin.

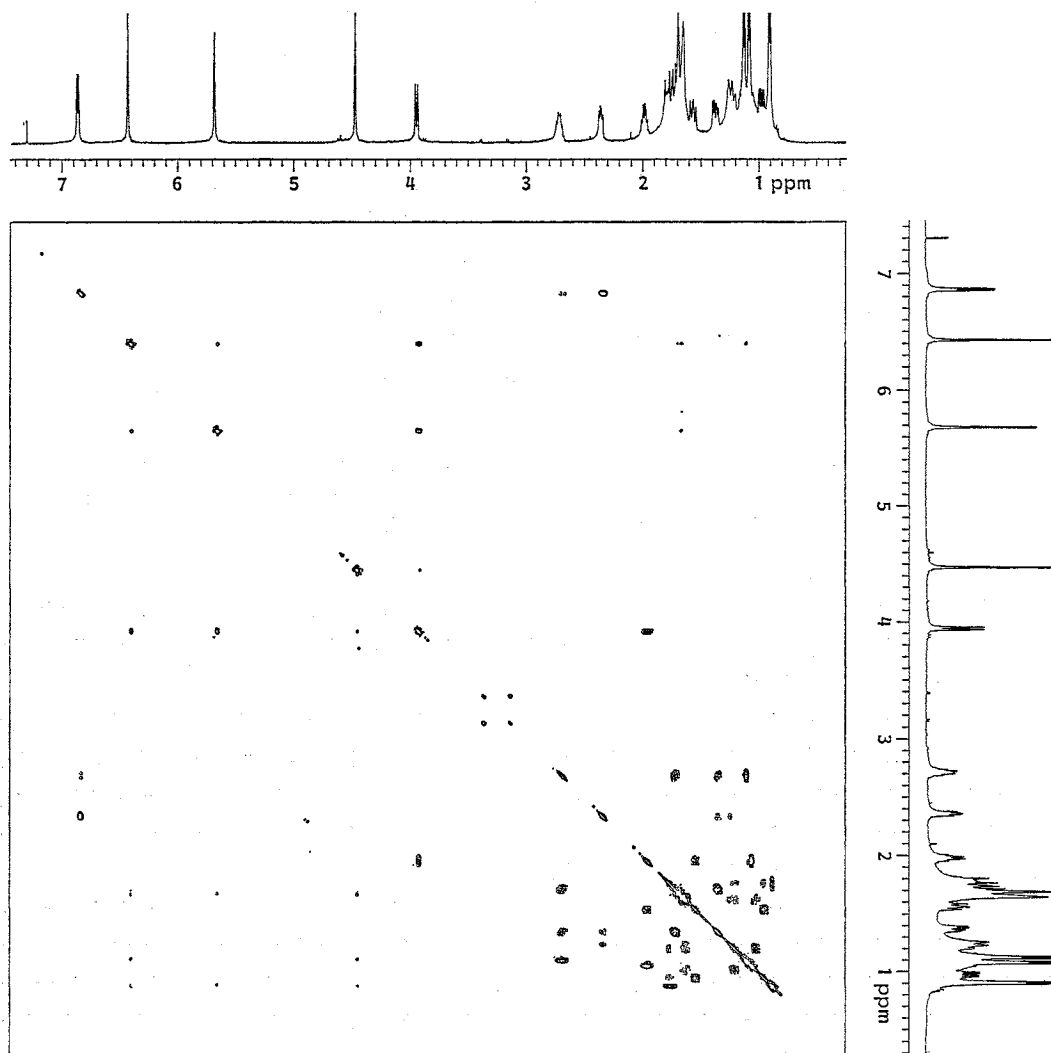
The fast atom bombardment mass spectra (FAB-MS) of okilactomycin showed quasi-molecular ion peaks at  $m/z$  417 ( $M+H$ )<sup>+</sup> in positive ion mode and at  $m/z$  415 ( $M-H$ )<sup>-</sup> in negative ion mode, and the electron ionization mass spectrum (EI-MS) showed the molecular ion peak at  $m/z$  416 ( $M^+$ ). The molecular formula was found to be  $C_{24}H_{32}O_6$  by the high resolution EI mass measurement (found: 416.2208, calcd: 416.2219) and elemental analysis.

Homonuclear shift correlation spectroscopy (COSY),<sup>2,3)</sup> heteronuclear shift correlation spectroscopy (<sup>1</sup>H-<sup>13</sup>C COSY)<sup>4-7)</sup> and long-range heteronuclear shift correlation spectroscopy (long-range <sup>1</sup>H-<sup>13</sup>C COSY)<sup>4-7)</sup> is given in Figs. 1~3, respectively. The structure of okilactomycin obtained by the present NMR study is shown in Fig. 4 with numbering scheme. The <sup>1</sup>H and <sup>13</sup>C chemical shifts are shown in Tables 1 and 2, respectively.

The <sup>13</sup>C NMR spectrum showed the expected 24 carbon signals. The refocused insensitive nuclear enhanced polarization transfer (INEPT) experiment<sup>8)</sup> assigned them to 3 carbonyl, 4 olefine, 4 methyl, 5 methylene, 6 methine and 2 quaternary carbon atoms.

In the COSY spectrum, an olefinic proton (17-H at 6.86 ppm) shows two cross peaks with resonances of methine protons (15-H at 2.70 ppm and 1-H at 2.36 ppm). <sup>1</sup>H-<sup>1</sup>H decoupling experiment revealed that the former cross peak is due to long-range coupling and the latter is due to vicinal coupling. The methine proton of 15-H shows further connectivity with methylene protons (14-H<sub>a</sub> and 14-H<sub>b</sub> at 1.37 and 1.72 ppm, respectively) and with methyl protons (19-H at 1.12 ppm). In the long-range <sup>1</sup>H-<sup>13</sup>C COSY spectrum, connectivity of 17-H proton is observed to a carbonyl carbon at 172.2 ppm (C-18), to a methine carbon at 27.0 ppm (C-15) and to a quaternary carbon at 84.8 ppm (C-13). The chemical shift of C-13 suggests that oxygen atom is attached to it. The C-13 quaternary carbon shows the connectivity with resonances of two methine protons (12-H at 4.47 ppm and 1-H at 2.36 ppm) and of a nonequivalent methylene proton 14-H<sub>a</sub>. And further, a long-range coupling between

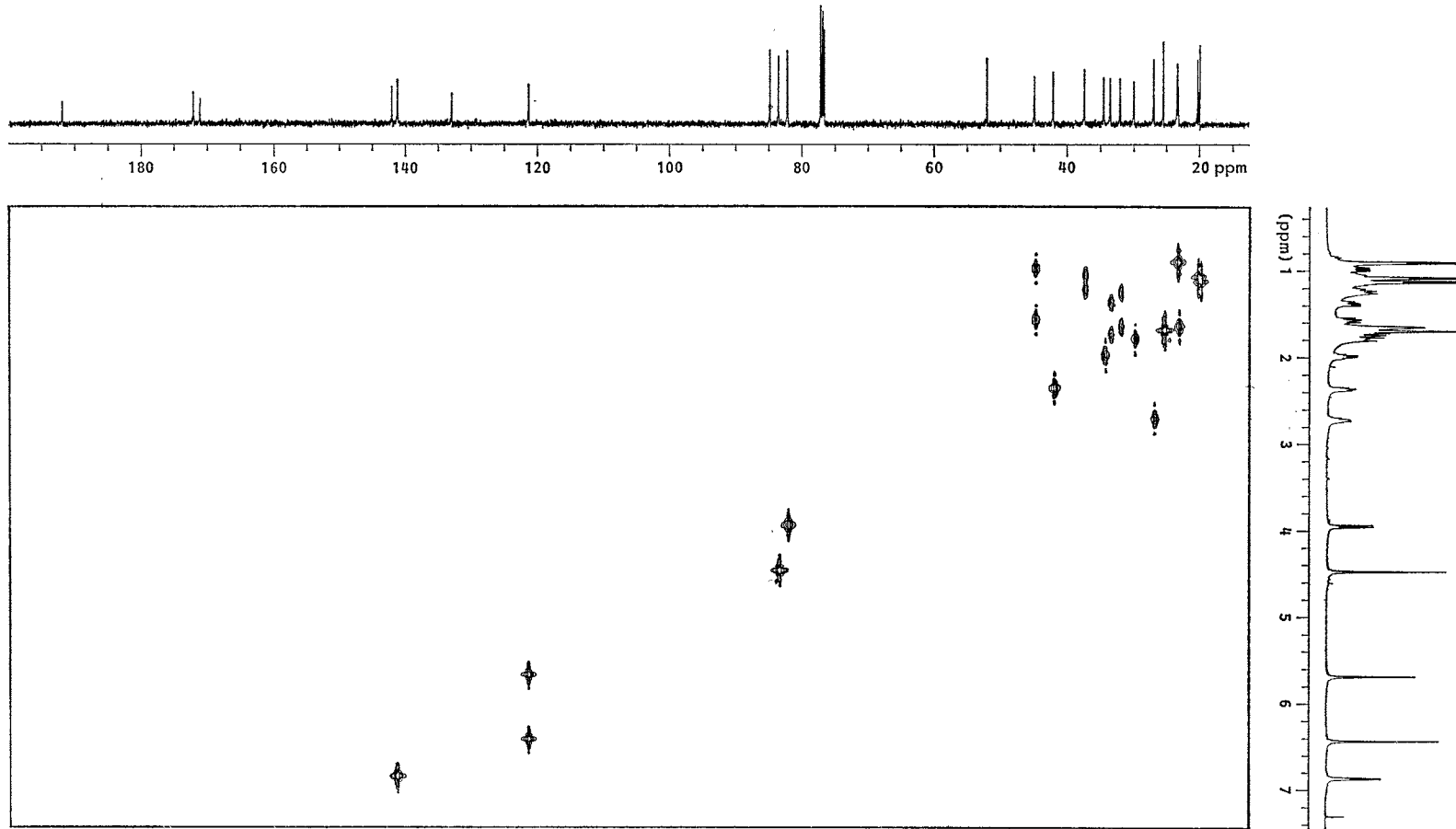
Fig. 1. COSY spectrum.



14-H<sub>a</sub> and 1-H were observed (Fig. 1). Therefore, a cyclohexene ring system containing a quaternary carbon attached to oxygen is indicated.

In the COSY spectrum, a methylene proton (2-H<sub>a</sub>) at 1.26 ppm, coupled with the methine proton (1-H), shows the cross peak with a resonance at 1.65 ppm. The signal at 1.65 ppm shows cross peak with resonances of methylene protons of 4-H<sub>a</sub> at 1.04 ppm and 4-H<sub>b</sub> at 1.23 ppm. No cross peak of the methylene proton of 2-H<sub>a</sub> at 1.26 ppm is observed with a resonance of the protons on C-4. The protons on C-2 and on C-4 are not vicinal. On the other hand, <sup>1</sup>H-<sup>13</sup>C COSY and INEPT experiments revealed that the peak at 1.65 ppm is due to three protons; one is a proton (2-H<sub>b</sub>) of the methylene and the others are equivalent methylene protons (3-H). Therefore, it is obvious that the C-3 methylene carbon is bonded to C-2 and C-4 carbon atoms. The connectivity by following carbon-carbon bonds can be easily traced to the terminal vinyl carbon of C-22 in the COSY spectrum.

Long-range <sup>1</sup>H-<sup>13</sup>C COSY correlation indicates connectivity of the carbonyl signal at 191.9 ppm



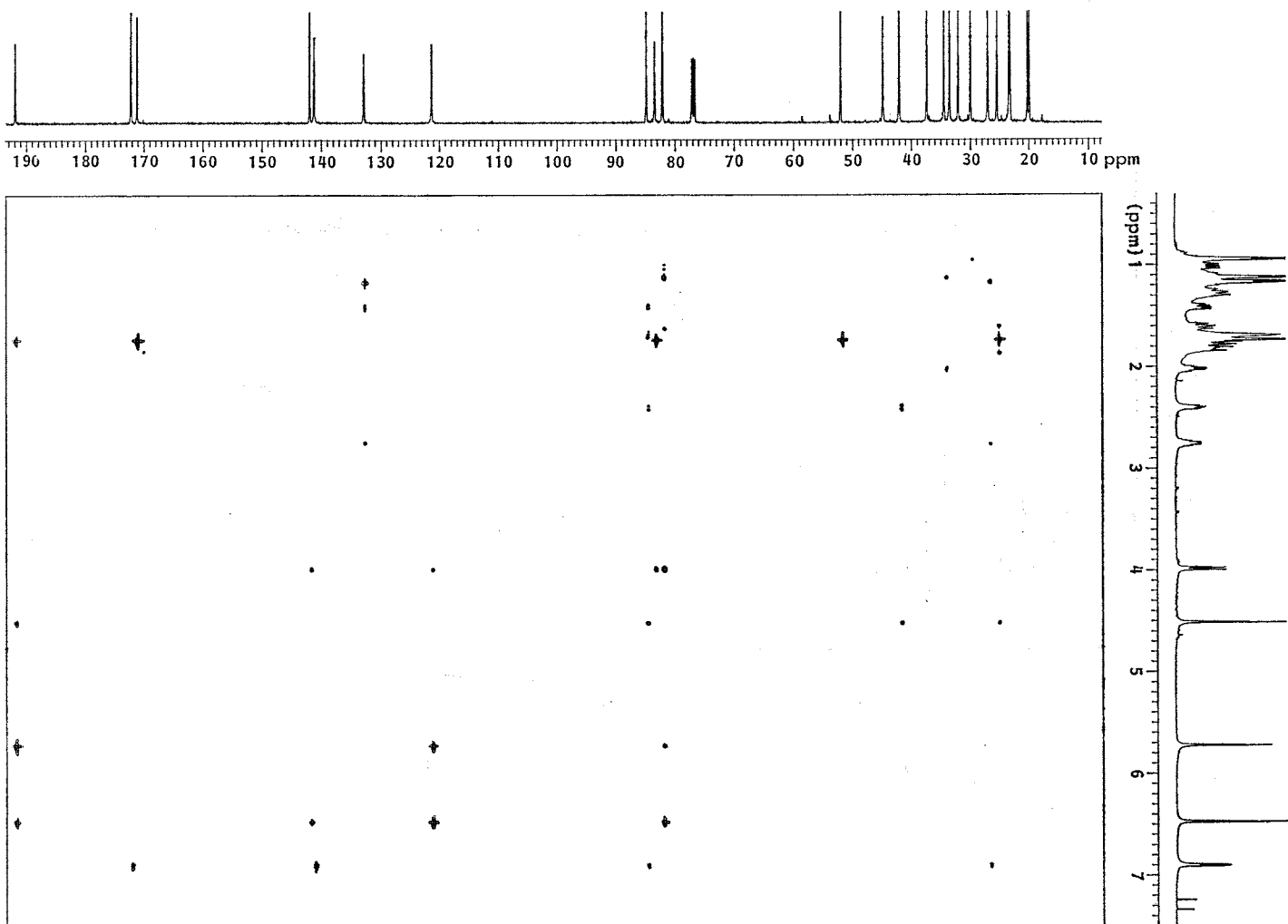


Table 1.  $^1\text{H}$  NMR chemical shifts of okilactomycin.

Proton No.	$\delta$ (ppm ( $J$ , Hz) in $\text{CDCl}_3$ )
1-H	2.36 m
2-H <sub>a</sub>	1.26 m
2-H <sub>b</sub>	1.65 m
3-H	1.65 m
4-H <sub>a</sub>	1.04 m
4-H <sub>b</sub>	1.23 m
5-H	1.78 m
6-H <sub>a</sub>	0.98 dd ( $J=13.7, 7.6$ )
6-H <sub>b</sub>	1.56 dd ( $J=13.7, 9.5$ )
7-H	1.98 m
8-H	3.94 d ( $J=9.6$ )
12-H	4.47 s
14-H <sub>a</sub>	1.37 m
14-H <sub>b</sub>	1.72 m
15-H	2.70 m
17-H	6.86 dd ( $J=6.1, 2.0$ )
19-H	1.12 d ( $J=6.8$ )
21-H	1.69 s
22-H <sub>a</sub>	5.68 s
22-H <sub>b</sub>	6.43 s
23-H	1.08 d ( $J=6.6$ )
24-H	0.91 d ( $J=6.5$ )
COOH	11.24 br s

(C-10) to a methyl proton (21-H at 1.69 ppm), a methine proton (12-H at 4.47 ppm) and terminal vinyl protons (22-H<sub>a</sub> at 5.68 ppm and 22-H<sub>b</sub> at 6.43 ppm). Further, connectivity of the methyl proton (21-H at 1.69 ppm) is observed to a carbonyl carbon (C-20 at 171.2 ppm), to a methine carbon (C-12 at 83.5 ppm) and to a quaternary carbon (C-11 at 52.0 ppm). The cross peak between 8-H and 12-H in the COSY spectrum and the chemical shifts of the carbon atoms indicate the C(8)-O-C(12) ether linkage. Therefore, tetrahydro- $\gamma$ -pyrone ring system is established.

All resonances in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra now have been assigned. Connectivity of oxygen atom at the C-13 quaternary carbon was not determined by the present NMR study. However, a consideration with the dreiding model have indicated the reasonable structure as shown in Fig. 4.

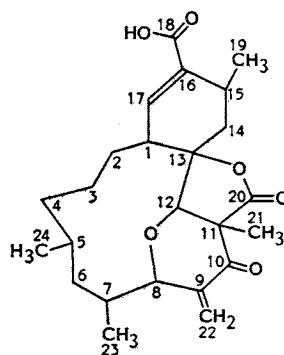
In order to confirm the molecular structure, X-ray crystallographic study has been carried out on a crystal containing methylene chloride as crystal solvent. The molecular structure of okilactomycin is shown in Fig. 5. The bond lengths and bond angles are in good agreement with expected values of the chemical structure, though the crystal solvent was not correctly assigned. The final atomic parameters, bond distances and bond angles, and Fo-Fc table were deposited to the Cambridge Crystallographic Center.

Okilactomycin has a 13-membered ring with the intra ether bridge forming tetrahydro- $\gamma$ -pyrone

Table 2.  $^{13}\text{C}$  NMR chemical shifts of okilactomycin.

Carbon No.	$\delta$ (ppm in $\text{CDCl}_3$ )
C-1	42.0 d
C-2	32.0 t
C-3	23.2 t
C-4	37.4 t
C-5	30.0 d
C-6	44.8 t
C-7	34.4 d
C-8	82.1 d
C-9	141.9 s
C-10	191.9 s
C-11	52.0 s
C-12	83.5 d
C-13	84.8 s
C-14	33.5 t
C-15	27.0 d
C-16	132.9 s
C-17	141.2 d
C-18	172.2 s
C-19	19.9 q
C-20	171.2 s
C-21	25.4 q
C-22	121.3 t
C-23	20.2 q
C-24	23.4 q

Fig. 4.



ring with the exomethylene at the  $\beta$ -position. The 13-membered ring is fused with the  $\gamma$ -lactone and the cyclohexene. The lactone and cyclohexene rings are connected at the spiro carbon. The cyclohexene ring has the methyl group and the carboxyl group, the carbonyl bond of which is in the anti-periplanar arrangement with the carbon-carbon double bond. Three exocyclic methyl groups are bonded to 13-membered ring.

In the EI-MS spectrum, the fragment ion  $m/z$  398 may be attributable to the  $(M-H_2O)^+$  ion peak. The base peak  $m/z$  125  $(C_7H_9O_2)^+$  may be derived from the tetrahydro- $\gamma$ -pyrone ring. Strong absorption band at 1790, 1760 and 1715  $cm^{-1}$  found in IR spectrum is assignable to  $\gamma$ -lacton, tetrahydro- $\gamma$ -pyrone and carboxylic acid, respectively.

### Experimental

#### Spectroscopic Studies

IR spectra were recorded on a Hitachi 260-50 IR spectrophotometer. FAB-MS and EI-MS were measured on a Jeol JMS-DX300 double focusing spectrometer using glycerol matrix and a Hitachi M-80 double focusing spectrometer, respectively.

$^1H$  and  $^{13}C$  NMR spectra were recorded on a Jeol JNM-GX500 (500 MHz for  $^1H$  and 125 MHz for  $^{13}C$ ) spectrometer; chemical shifts are given in ppm relative to TMS (0 ppm) as an internal standard and coupling constants ( $J$ ) are recorded in Hz.

#### X-Ray Crystallographic Study

Colorless prismatic crystals were deposited in methylene chloride solution. Elementary analysis of the crystals showed the presence of methylene chloride as crystal solvent; *Anal Calcd* for  $C_{24}H_{32}O_6 \cdot CH_2Cl_2$ : C 59.88, H 6.83, Cl 14.14. *Found*: C 59.63, H 6.76, Cl 14.04. The density was measured by the floatation method in *n*-hexane and carbon tetrachloride mixture.

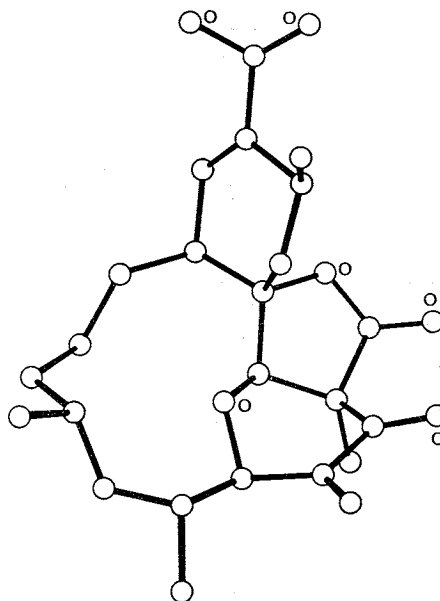
A crystal of approximate dimensions of  $0.3 \times 0.3 \times 0.2$  mm<sup>3</sup> was used for data collection on a Rigaku AFC-5R diffractometer with  $CuK\alpha$  radiations ( $\lambda = 1.54184$  Å). Accurate unit cell dimensions were derived from  $2\theta$  values of 25 high-angle reflections. The crystal data are as follows:  $C_{24}H_{32}O_6 \cdot CH_2Cl_2$ ,  $FW = 501.45$ , monoclinic, space group C2,  $a = 20.609(3)$ ,  $b = 10.214(1)$ ,  $c = 12.372(1)$  Å,  $\beta = 92.84(2)^\circ$ ,  $Z = 4$ ,  $D_{measured} = 1.28$  gcm<sup>-3</sup> and  $D_{calc} = 1.280$  gcm<sup>-3</sup>.

Intensities were measured in the  $2\theta/\omega$  scan mode. Five reference reflections monitored every 50 reflections showed no significant intensity deterioration. Corrections were made for Lorentz and polarization factors but not for absorption.

The phases were derived by the MULTAN84<sup>9)</sup> with 328 largest E's ( $>1.39$ ). A partial skeleton was found on the electron density map derived from the phase set with the largest combined figure of merit. Subsequent electron density calculations gave the complete molecular skeleton. The atomic identification is based on the results obtained by the NMR study. Their positional and thermal parameters were refined by the block-diagonal least-squares calculations. Hydrogen atoms were located appropriately by geometrical calculation and fixed. A difference Fourier synthesis showed electron density peaks around the 2-fold symmetry axis at  $(0, y, 0)$  and  $(0, y, 1/2)$ . The electron density peaks

Fig. 5. Perspective drawing of okilactomycin.

The relative configuration is  $1S^*$ ,  $5R^*$ ,  $7R^*$ ,  $8R^*$ ,  $11R^*$ ,  $12R^*$ ,  $13S^*$ ,  $15S^*$ , the numbering scheme shown in Fig. 4.



suggesting crystal solvent were not correctly assigned because of disorder but included in the refinement in order to refine the okilactomycin molecule. The final R value was 0.10 for 2040 reflections with  $|F_o| > 3\sigma(|F_o|)$  and the maximum shift of parameters in the last cycle was  $0.4\sigma$ .

Atomic scattering factors used were taken from "International Tables for X-ray Crystallography".<sup>10)</sup>

#### Acknowledgments

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