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Molecular Structure of a Novel Antitumor Antibiotic Leinamycin

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The structure of a novel antitumor antibiotic leinamycin isolated from *Streptomyces sp.* was determined unequivocally by X-ray analysis. The molecule has a unique 1,3-dioxo-1,2-dithiolane moiety being connected to an 18-membered lactam ring through a spiro carbon.

A novel antibiotic leinamycin is effective against murine experimental leukemia P388(ip), showing significant increase in life span(ILS 57%) at a dose of 0.38 mg/kg(ip).[1] It is effective against sarcoma 180(sc), exhibiting the ratio of median tumor volume(T/C 41%) at a dose of 1 mg/kg(iv). The compound also shows a broad antimicrobial activity against Gram-positive and Gram-negative bacteria. The LD_{50} value of it is 2.8 mg/kg(iv) in mouse. In addition to the high biological activity the mode of action of this unique compound is noteworthy. Hara et al.[2] have investigated the effect of the antitumor agent on DNA. In vitro leinamycin causes single-strand cleavage of supercoiled double-helical pBR322 DNA in the presence of thiol cofactors. Scavengers of oxygen radical did not suppress the DNA cleaving activity. In order to disclose the structure of the novel compound we have undertaken the X-ray analysis.

It was quite difficult to obtain single crystals of leinamycin. We have tried more than 300 combinations of various solvent and finally found out a solvent system of CH_2Cl_2:$CHCl_3$=3:10. Only this system gave the marginal crystals. The crystals were grown at 5 °C by controlled evaporation. Each batch with ca.2 mg gave only a few single crystals. The crystals thereby obtained were very small and usually cracked during crystal growth. After more than three hundred trials only one crystal with a sufficient size was obtained. Using the sole crystal we have measured the diffraction intensities. Crystal data are as follows: $C_{22}H_{26}N_2O_6S_3 \cdot 2CHCl_3 \cdot CH_2Cl_2$, Mr=834.09, monoclinic, space group C2, Z=8, a=23.99(1), b=11.369(5), c=22.766(5) Å, β=99.12(4)°, V=6131 Å^3, Dx=1.34 gcm^{-3}, F(000)=426. A needle crystal of approximately 0.4x0.3x0.15 mm was mounted on an Enraf-Nonius CAD-4 diffractometer with the graphite-monochromated Cu Kαradiation (μ=49.6 cm^{-1}) at 10 °C. Intensities of 3974 reflections with 2θ<120° were measured. The crystal decayed quickly by X-ray radiation and X-ray diffraction from the crystals were very weak. The total loss in intensities of three standard reflections was 33.8% in total exposure time of 25.6 h. On the basis that $I>3\sigma(I)$ 1507 reflections were considered as observed. The structure was solved by direct methods using MITHRIL[3] and DIRDIF .[4] Successive applications of DIRDIF and Fourier syntheses finally completed the molecular skeleton. There are two crystallographically independent molecules in an asymmetric unit. Two chloroform and one methylene-chloride molecules were found in the process of structure determination. The final difference Fourier map was quite noisy and more solvent molecules with less occupancies may exist . The structural parameters were refined

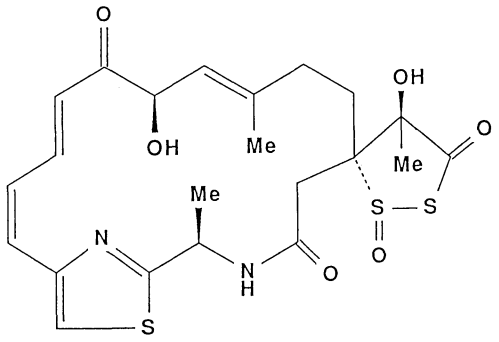

Fig.1. Chemical Structure of Leinamycin.

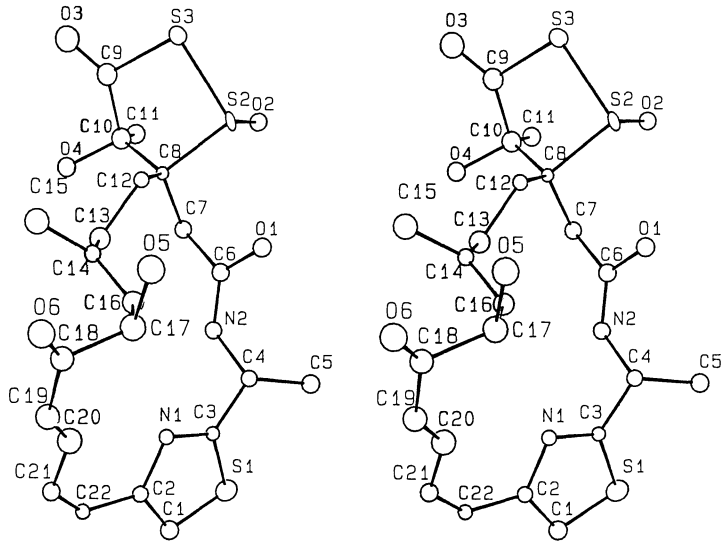

Fig.2. An ORTEP II Drawing of One of the Crystallographically Independent Molecules.

by full-matrix least-squares with CAD-4 structure determination package.[5] The final refinement converged at an R factor of 0.091 for 1438 observed reflections($\sin\theta/\lambda<0.45$). The absolute configuration could not be determined uniquely . The chemical structure and an ORTEP II drawing[6] of one of the two independent molecules are shown in Figs.1 and 2, respectively.

One of the most characteristic features is a five-membered ring which can be identified as 1,3-dioxo-1,2-dithiolane. This five-membered ring has not been found in any natural products so far. This unique partial structure is connected to an eighteen-membered lactam ring with a thiazole ring through a spiro bond. 1,3-Dioxo-1,2-dithiolane ring adopts an envelope conformation with the spiro carbon atom at a flap position. The flap bends sharply with the flap carbon atom being deviated by 0.76 Å in average from the least-squares planes defined by other four atoms in the ring. The thiocarbonyl oxygen atom is axial and the carbonyl oxygen atom is equatorial-oriented. The thiazole ring is planar.

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