ABSOLUTE CONFIGURATION OF EPIDERSTATIN, A NEW GLUTARIMIDE ANTIBIOTIC PRODUCED BY *Streptomyces pulveraceus*

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Epiderstatin was isolated as a potent inhibitor of the signal transduction induced by EGF in quiescent Balb/MK cells[1]. The chemical structure of the compound was proposed as 4-[3-((*Z*)-3,5-dimethyl-2-oxopiperidine-6-ylidene)-2-oxopropyl]-2,6-piperidinedione on the basis of spectroscopic evidence[2]. On comparison with other glutarimide antibiotics, epiderstatin is unique in containing a vinylogous amide. However, the relative configuration at C-3 and C-5 in its structure could not be determined by the ^{1}H NMR spectral analysis because the methine proton signals of 3-H, 5-H and 10-H overlapped.

We report herein the relative configuration of epiderstatin and also its absolute configuration (Fig. 1) which were determined by means of X-ray analysis, and COSMIC force-field energy calculation and circular dichroism (CD) analysis, respectively.

Results and Discussion

The molecular structure of epiderstatin determined by X-ray crystallography (Fig. 2) showed that the relative configurations at C-3 and C-5 could be either *S*/*S* or *R*/*R*.

To determine the absolute configuration of epiderstatin, COSMIC force-field calculation and CD analysis were carried out. On the basis of the X-ray data, the steric energies of the two hypothetical conformations of epiderstatin in COSMIC force-field were calculated with the analytical program Nemesis (Fig. 3)[3]. The steric energy of the 5-CH_3 axial conformer (18.0 kcal/mole) is thermodynamically lower than that of the 3-CH_3 axial conformer (22.3 kcal/mole), therefore, the 5-CH_3 axial conformer, which has a pseudoaxial methyl at the C-5 position, is more stable than the 3-CH_3 axial conformer. The stability of the 5-CH_3 axial conformer could be justified by the van der Waals repulsion between 5-CH_3 and 7-H. This conformation in solution was also confirmed from the NOE experiments which show that the 5-CH_3 exists predominantly in a pseudoaxial position, because the NOE between 5-H and 7-H (5.5%) is larger than that between 5-CH_3 and 7-H (0.7%)[2]. Thus, the 5-CH_3 axial conformation in solution was deduced from both COSMIC force-field calculation and X-ray crystallography in combination with NOE experiments.

The circular dichroism (CD) curve (Fig. 4) of the compound in CH_3OH showed a positive Cotton effect ($\Delta\varepsilon$ +0.165) at 285 nm. An application of the allylic axial chirality approach[4] for the long-wavelength $\pi-\pi^*$ transition Cotton effect of the cisoid conjugated enone system indicated that the absolute configuration of C-5 is *S*. The shift of the *K* band to longer wavelength is probably due to the existence of the electron-donating amide-nitrogen, N-1, on which the unshared electron pair interacts

Fig. 1. Absolute configuration of epiderstatin.

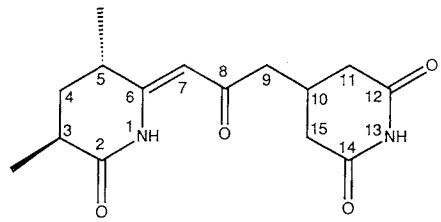

Fig. 2. Molecular structure of epiderstatin.

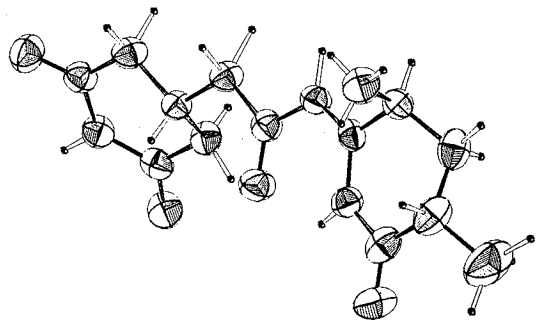

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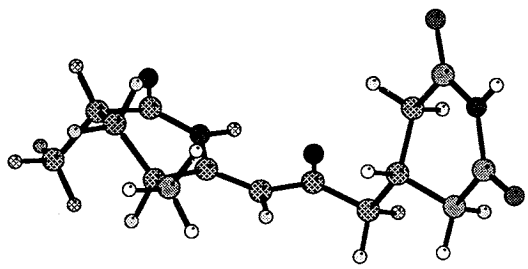
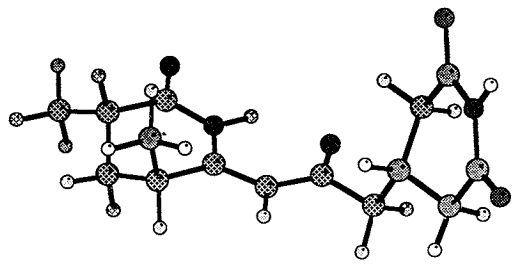
Fig. 3. Steric conformation of epiderstatin estimated by the COSMIC force-field method.

3-CH_3 axial



Total energy: 22.3 kcal/mole

5-CH_3 axial



Total energy: 18.0 kcal/mole

with the delocalised π orbitals of the conjugated enone. It was thus concluded that the absolute configuration of epiderstatin is (3*S*,5*S*) as shown in Fig. 1. This configuration corresponds to that of cycloheximide[5], which suggests that the both compounds are derived from a common biosynthetic precursor.

Two independent synthesis of (±)-epiderstatin were recently reported[6,7], and the Pfizer group[6] indicated the C-3/C-5 relative stereochemistry as *trans*.

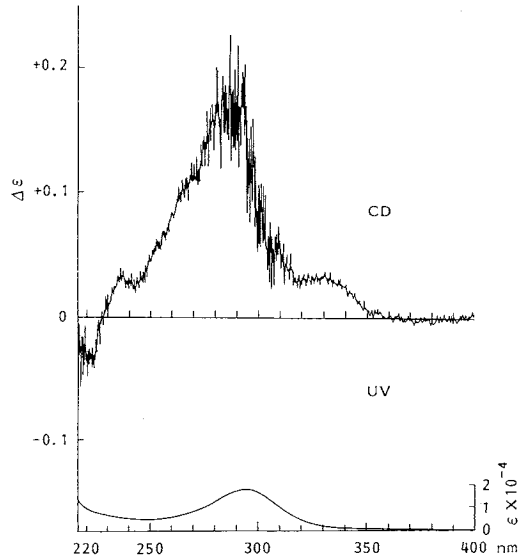
Experimental

Epiderstatin was isolated from the culture fluid of *Streptomyces pulveraceus* subsp. *epiderstagenes* as described in ref 1. Crystals were obtained from methanol-water (1:1) in a refrigerator.

Crystal Data

$C_{15}H_{20}N_2O_4$, $Mw=292.33$, orthorhombic, $a=10.938(2)$, $b=23.871(6)$, $c=5.851(1)$ Å, $V=1527.7(6)$ Å^3, space group $P2_12_12_1$, $Z=4$, $Dx=1.271$ g/cm^3, μ(Mo−Kα)$=0.87$ cm^{-1}, $F(000)=624$. Lattice parameters were determined from 20 reflections with $20°<2\theta<23°$.

Fig. 4. CD curve of epiderstatin.



Data Collection and Processing

A transparent colorless prism crystal (0.14×0.22×0.80 mm) was mounted on a Rigaku AFC-4 diffractometer. Intensity data were measured using graphite-monochromated Mo−Kα radiation, $\lambda=0.7073$ Å, in the ω-scan mode (0 to 30°) and $\omega-2\theta$ scan mode ($30°<2\theta$). The three standard reflections showed no significant deterioration. The intensities were corrected for Lp factors. Within the range of $2\theta\leqq55°$, 1,307 reflections were measured and 1,239 unique reflections obtained with $|F_o|>3\sigma(|F_o|)$.

Structure Analysis and Refinement

The structure of epiderstatin was solved by the direct methods using the program MULTAN78[8] and refined by the block-diagonal least-squares. All H atom positions were located from a difference Fourier synthesis. All non-hydrogen atoms were refined anisotropically and H atoms isotropically. Both final R and Rw values were 0.046. Crystallographic calculations were performed on a FACOM M-780 computer using UNICS-III program system[9]. The source of scattering factor data was given by International Tables for X-ray Crystallography (1974)[10].

Calculation of Steric Energy of Epiderstatin

The steric conformations of epiderstatin were built up by three dimensional graphics on a Macintosh computer. On the basis of these structure, each steric energy was calculated by the COSMIC force-field method using a Nemesis program (Oxford Molecular Ltd.)[3].

Determination of Circular Dichroism (CD)

The CD spectrum of epiderstatin was determined with a JASCO J-20 Automatic Recording Spectropolarimeter at a concentration of 50 μg/ml in methanol. The scanning conditions were as follows: scanning speed, 4 mm/minute; temperature, 25°C; cell length, 10 mm; scale, 1.0 mm°/cm.

References

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