THE STRUCTURE OF PYRIZINOSTATIN

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In a previous communication[1], we have described the isolation, physico-chemical properties and biological properties of pyrizinostatin (Fig. 1), a new inhibitor of pyroglutamyl peptidase (PG-peptidase). In this paper, the structure determination of pyrizinostatin is reported.

Pyrizinostatin was obtained as colorless crystals, and its molecular formula was established as $C_{11}H_{15}N_5O_4$ by HRFAB-MS and elemental analysis. The IR spectrum exhibited strong absorption at 1680 cm^{-1} suggesting the presence of amide bond in the molecule (Fig. 2). The UV spectra showed a maximum at 280 nm (ε 4,600) in MeOH.

The ^{13}C and ^1H NMR data for pyrizinostatin are summarized in Table 1. In the ^{13}C NMR spectrum, the signals of 11 carbons were resolved and assigned to four methyl carbons, one methylene carbon, one quaternary carbon and five quaternary sp^2 carbons by DEPT experiment. In the ^1H NMR spectrum, characteristic signals due to four isolated methyl groups were observed, and the three methyl groups could be assigned to *N*-methyl protons based on their ^{13}C and ^1H chemical shifts. One exchangeable proton signal (δ 5.75) was attributed to be NH proton because it gave positive color reaction with Greig-Leaback reagent[2]. All of the proton signals of pyrizinostatin was located, and then the proton-carbon correlations were obtained by a heteronuclear multiple-bond correlation (HMBC)[3] experiment and assignment of ^1H and ^{13}C NMR spectra were elucidated. The summary of the ^1H-^{13}C long range coupling by HMBC measurements on pyrizinostatin is shown in Fig. 3.

The structure of pyrizinostratin was determined by crystal X-ray diffraction analysis. A colorless

Fig. 1. Structure of pyrizinostatin.

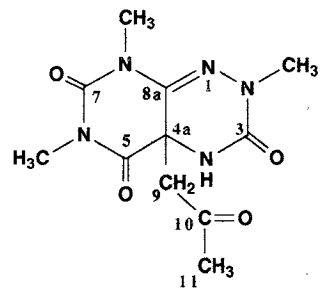


Fig. 2. IR spectrum of pyrizinostatin (KBr).

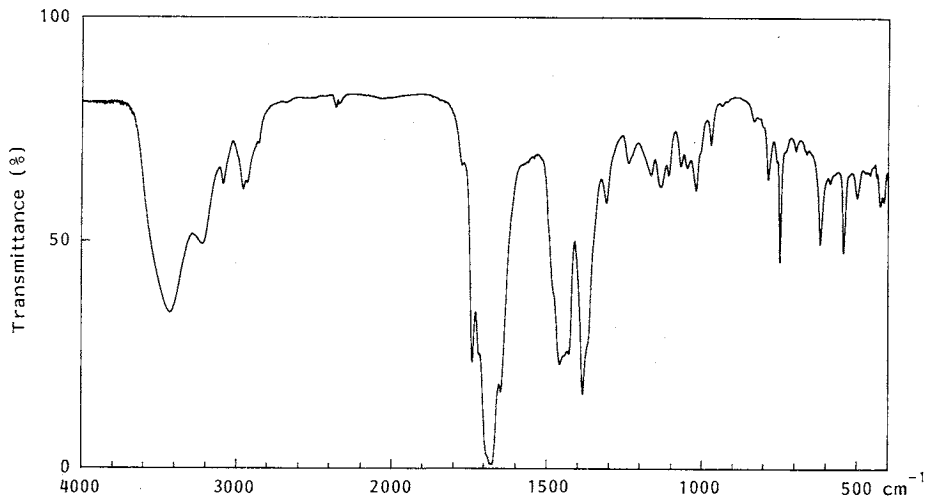
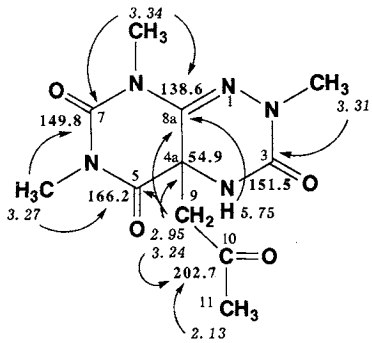


Table 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data of pyrizinostatin in $CDCl_3$.

Position	^{13}C	M	^{1}H (J=Hz)
N2-CH_3	37.0	q	3.31
3	151.5	s	—
N4-H	—	—	5.75
4a	54.9	s	—
5	166.2	s	—
N6-CH_3	28.8	q	3.27
7	149.8	s	—
N8-CH_3	30.4	q	3.34
8a	138.6	s	—
9	49.6	t	2.95 (16.0),
	—	—	3.24 (16.0)
10	202.7	s	—
11	30.8	q	2.13

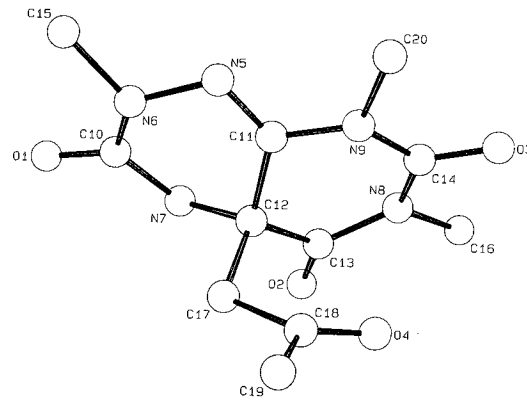
Chemical shifts in ppm from TMS.
M: Multiplicity.

Fig. 3. HMBC data summary for pyrizinostatin.



prism crystal of $C_{11}H_{15}N_5O_4$ having approximate dimensions of $0.2 \times 0.2 \times 0.1$ mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated CuK_α radiation and a 13 KW rotating anode generator. Cell constants were $a=20.352(3)$, $b=9.156(2)$, $c=15.322(3)$Å, $V=2720.6(9)$Å^3, $b=107.66(1)$, $Z=8$ and the calculated density is 1.373 g/cm^3. Based on the systematic absences, the space group was determined to be C2/c (#15). The data were collected at room temperature using the $\omega-2\theta$ scan technique to a maximum 2θ value of 120.3°. Omega scan of several intense reflections, made prior to data collection, had an average width at half-height of 0.19° with a take-off angle of 6.0°. Scans of $(1.25+0.30\ tan\ \theta)$ were made at a speed of 16.0°/minute. The equivalents were merged ($R_{int}=0.049$), finally 1,838 reflections were obtained. The structure was solved by SHELXS[4]. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 473 observed reflections ($I>3.000(1)$) and 181 variable parameters and converged (largest parameter shift was 2.42 times its end) with $R=10.2\%$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.43 and -0.38 e$^-$/Å^3, respectively. All calculations were performed using the TEXSAN crystallographic software package of Molecular Structure Corporation. A PLUTO[5] drawing of the molecule is shown in Fig. 4.

Fig. 4. Molecular structure of pyrizinostatin.



Therefore, the structure of pyrizinostatin was determined to be 2,4,4a,8-tetrahydro-2,6,8-trimethyl-4a-(2-oxopropyl)-pyrimido[5,4-*e*]-1,2,4-triazine-3,5,7(6*H*)-trione.

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