EPIDERSTATIN, A NEW INHIBITOR OF THE MITOGENIC ACTIVITY INDUCED BY EPIDERMAL GROWTH FACTOR

II. STRUCTURE ELUCIDATION

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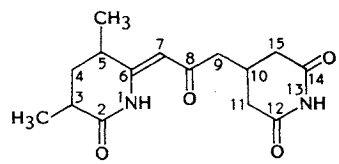
The structure of a novel antibiotic, epiderstatin, was determined as 4-[3-((*Z*)-3,5-dimethyl-2-oxopiperidin-6-ylidene)-2-oxopropyl]-2,6-piperidinedione by spectroscopic analyses of ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H correlation spectroscopy (COSY), ^{13}C-^{1}H COSY, heteronuclear multiple bond correlation spectroscopy, UV and IR spectra. The antibiotic belongs to the glutarimide antibiotics, however, the characteristic feature of epiderstatin is that it has a piperidone ring instead of a cyclohexanone ring.

Recently, we isolated a new antibiotic, epiderstatin, from the culture fluid of a streptomycete, *Streptomyces pulveraceus* subsp. *epiderstagenes*[1]. The biological activity of this antibiotic is very interesting as it inhibits the incorporation of [^{3}H]thymidine into epidermal growth factor (EGF)-stimulated quiescent cells and changes the morphology of ^{ts}*src*-transformed NRK cells to normal cells. Taxonomy, isolation and physico-chemical properties were reported in a previous paper[1]. In this paper, we propose the structure of epiderstatin as 4-[3-((*Z*)-3,5-dimethyl-2-oxopiperidin-6-ylidene)-2-oxopropyl]-2,6-piperidinedione (Fig. 1).

Fig. 1. Structure of epiderstatin.



Experimental

Instrumental Analyses

^{1}H NMR, ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear multiple bond correlation spectroscopy (HMBC), and ^{13}C-^{1}H COSY spectra of epiderstatin were obtained on a Jeol GSX-500 spectrometer at 500 MHz in $CDCl_3$. ^{13}C NMR spectra were measured on a Jeol FX-100 fourier transform (FT)-NMR spectrometer at 25 MHz in $CDCl_3$. Nuclear Overhauser effect (NOE) experiments were run on a Jeol GX-400 spectrometer at 400 MHz in $CDCl_3$.

Epiderstatin

Epiderstatin (9.2 mg) was isolated from 72 liters of culture broth of *S. pulveraceus* subsp. *epiderstagenes* as described in the previous paper[1]. Physico-chemical properties were also described.

Methylation of Epiderstatin

Epiderstatin (2.0 mg) was treated with a large excess of diazomethane in diethyl ether and allowed to stand at room temperature overnight. Monomethylated epiderstatin was appeared homogeneous on TLC (Kieselgel 60F_{254}, 95 : 5, chloroform - methanol, Rf 0.59) and isolated by TLC and obtained as an amorphous powder in about 70% yield.

Results and Discussion

Characteristic UV absorption (295 nm, ε 16,700) of epiderstatin indicated the presence of β-acylamino-α,β-unsaturated ketone[2]. The IR spectrum suggested the presence of an α,β-unsaturated ketone and amide and/or imide (3430, 1725, 1701, 1643 and 1584 cm^{-1}).

The ^1H NMR spectrum of epiderstatin showed twenty signals due to two methyl, four methylene, four methine and two NH groups. The ^{13}C NMR spectrum of the antibiotic exhibited fifteen signals consisting of two methyl, four methylene, four methine, two olefinic and four carbonyl carbons.

The correlation between protons and carbons revealed by the ^{13}C-^1H COSY spectrum of epiderstatin is presented in Table 1. The partial structures A and B shown in Fig. 2 were deduced from scalar couplings among 3-CH_3, 3-H, 4-H, 5-H and 5-CH_3, and 9-H, 10-H, 11-H and 15-H, respectively, in the ^1H-^1H COSY spectrum.

In the HMBC spectrum of epiderstatin, long-range H-C couplings were observed between 3-CH_3 and C-2, C-3, C-4; 5-CH_3 and C-4, C-5, C-6; 7-H and C-5, C-8; 9-H and C-8, C-10, C-11, C-15; 11-H and C-10, C-12; 15-H and C-10, C-14 (Fig. 3). From these data, the structure of epiderstatin was deduced as shown in Fig. 1.

The olefinic *Z* configuration was determined by an NOE experiment. By irradiating 7-H, an NOE was observed on 5-H, 5-C*H*$_3$ and 9-H. Contrary, by irradiating 5-C*H*$_3$, an NOE was observed on 4-H, 5-H and 7-H.

Methylation of epiderstatin by diazomethane gave a monomethylated epiderstatin with *m/z* 306 in electron impact (EI)-MS spectrum. The ^1H NMR spectrum showed that 13-NH (7.98 ppm) of epiderstatin was substituted by NCH_3 at 3.15 ppm. The existence of an imide group was confirmed by a long-range coupling between the NCH_3 protons and both C-12 and C-14 in the HMBC spectrum. The proton on N-1 is considered to be involved in intramolecular hydrogen bonding with the 8-carbonyl, explaining its resistance toward methylation. The stereochemistry at C-3 and C-5 is under study.

Table 1. ^{13}C-^1H COSY spectrum of epiderstatin (in $CDCl_3$).

Position	δ_C (ppm)	δ_H (ppm)	Multiplicities	
1	—	11.57	s, 1H	NH
2	173.5	—		C=O
3	32.3	2.70	m, 1H	CH
4	34.0	1.83	m, 2H	CH_2
5	31.0	2.66	m, 1H	CH
6	160.3	—		C=
7	99.2	5.20	s, 1H	CH=
8	197.6	—		C=O
9	46.9	2.48	d, 2H	CH_2
10	26.6	2.73	m, 1H	CH
11	37.4	2.36, 2.75	m, dd, 2H	CH_2
12	171.6	—		C=O
13	—	7.98	s, 1H	NH
14	171.6	—		C=O
15	37.4	2.36, 2.75	m, dd, 2H	CH_2
3-CH_3	16.3	1.28	d, 3H	CH_3
5-CH_3	19.7	1.31	d, 3H	CH_3

Fig. 2. Partial structures of epiderstatin estimated from the ^1H-^1H COSY spectrum.

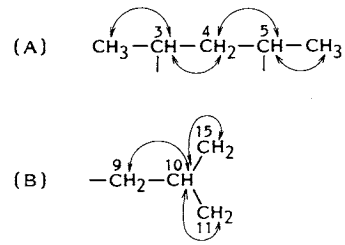
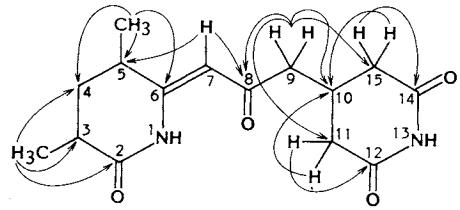


Fig. 3. Long range C-H coupling observed in the HMBC spectrum of epiderstatin (in $CDCl_3$).



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References

1) OSADA, H.; T. SONODA, H. KUSAKABE & K. ISONO: Epiderstatin, a new inhibitor of the mitogenic activity induced by epidermal growth factor. I. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 42: 1599~1606, 1989
2) Sadtler Research Laboratories, Inc. (*Ed.*): *N*-(3-Oxo-1-cyclohexen-1-yl)acetamide. *In* The Sadtler Standard Spectra. U-V IX. p. 9185, Sadtler Research Laboratories, Inc., 1965