STUDIES ON INHIBITORS OF RAT MAST CELL DEGRANULATION PRODUCED BY MICROORGANISMS

II. STRUCTURE ELUCIDATION OF EUROCIDINS D AND E

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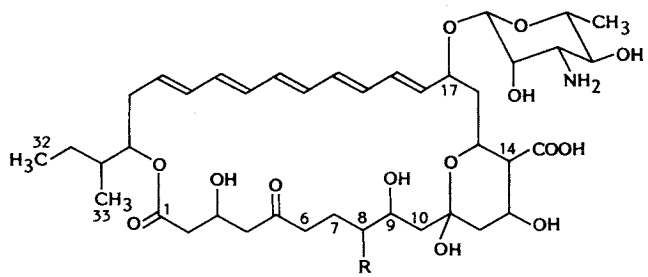
(Received for publication December 5, 1989)

The planar structures of new eurocidin related compounds, eurocidins D and E, were elucidated from ^{1}H-^{1}H shift correlated 2D NMR spectra and other NMR data. All protons in the molecules were assigned. Eurocidins D and E have novel pentaenic structures of eurocidin family.

In the preceding paper[1], screening of microorganisms, isolation and physico-chemical properties of new eurocidin related compounds, eurocidins C, D and E have been described. These compounds were isolated from the culture broth of *Streptoverticillium eurocidicum* IFO 13491 as the potent inhibitors of mast cell degranulation induced by compound 48/80.

We report here the structure elucidation of eurocidins D and E, the major components of them, from the NMR data and other physico-chemical properties.

Fig. 1. Structures of eurocidins D and E.



Eurocidin D R=OH
Eurocidin E R=H

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Results and Discussions

^{13}C NMR Spectra

The ^{13}C NMR data of eurocidins D and E are shown in Table 1. The measurement of multiplicity was carried out with DEPT experiments ($\theta=45°$, 90° and 135°). It was estimated from elemental analysis and the mass spectral data in the preceding paper[1] that the carbon number of eurocidins D and E is 40. The signals of 38 and 39 carbons in eurocidins D and E, respectively, were observed in their ^{13}C NMR spectra because of mutual superposition in chemical shifts of conjugated double bond carbons at

Table 1. ^{13}C NMR data of eurocidins D and E and their comparison with those of amphotericins A and B.

Carbon	Chemical shift (ppm), multiplicity			
	Eurocidin D	Eurocidin E	Amphotericin A[a]	Amphotericin B[a]
Ketone	208.7 s	208.0 s		
COOH	176.8 s	176.8 s	176.1	177.6
Lactone	169.9 s	169.9 s	170.3	170.1
=CH	136.6 d	136.6 d		
=CH	133.7 d	133.6 d		
=CH	133.5 d	133.5 d		
=CH	d	d		
=CH	132.5 d	132.5 d		
=CH	131.3 d	131.4 d	129~136	
=CH	d	131.2 d		
=CH	130.9 d	131.1 d		
=CH	129.8 d	129.8 d		
=CH	128.6 d	128.5 d		
Hemiketal	97.1 s	97.0 s	97.0	97.1
Acetal	95.9 d	95.8 d	97.3	95.9
CH	75.0 d	74.9 d		
CH	74.2 d	74.1 d		
CH	72.7 d	72.6 d		
CH	72.5 d			
CH	70.7 d	69.9 d		
CH	70.0 d	67.9 d	66~76	
CH	68.0 d	67.5 d	55~60	
CH	65.6 d	65.6 d	38~39	
CH	65.5 d	65.5 d		
CH	64.1 d	63.9 d		
CH	58.3 d	58.2 d		
CH	56.1 d	56.1 d		
CH	38.5 d	38.4 d		
CH_2	48.9 t	49.0 t		
CH_2	44.7 t	46.0 t		
CH_2	43.5 t	44.6 t		
CH_2	42.6 t	43.7 t		
CH_2	41.4 t	43.3 t	40~45	
CH_2	36.6 t	37.9 t	28~35	
CH_2	35.1 t	36.6 t		
CH_2	26.3 t	35.1 t		
CH_2	25.3 t	25.2 t		
CH_2		18.6 t		
CH_3	17.9 q	17.9 q		
CH_3	14.1 q	14.1 q		
CH_3	11.4 q	11.4 q		

[a] In literature[3].

128~137 ppm region. The numbers of multiplicity of carbons were >C<; 4, $-\overset{|}{C}H$; 24, $>CH_2$; 9 and CH_3; 3 for eurocidin D and >C<; 4, $-\overset{|}{C}H$; 23, $>CH_2$; 10 and CH_3; 3 for eurocidin E, respectively. Consequently, it was found that one of methylenes of eurocidin E changed to a methine of eurocidin D, probably by the substitution of a hydrogen atom with a hydroxyl group.

HORII *et al.*[2] reported that the partial structure of eurocidin A, which is purified from Eurocidin-T produced by *Streptomyces albireticuli*, contained 40 carbon atoms, pentaenic double bonds, a 30-membered lactone ring, a secondary butyl side chain, a carboxyl group, a mycosamine moiety and some hydroxyl groups. The structures of eurocidins D and E were similar to that of eurocidin A, with regard to the total carbon number and two of four singlet carbon signals (^{13}C NMR data). The two singlet carbon signals among four ones were defined as a lactone carbon and a carboxyl one. The comparison of the ^{13}C and ^1H NMR data of eurocidins D and E (Tables 1 and 2) with those of amphotericins A and B[3,4], heptaene macrolide antibiotics, suggests that the remaining two singlet carbon signals correspond to those of a ketone and a hemiketal.

^1H NMR and ^1H-COSY Spectra

The assignment of the protons of eurocidins D and E molecules is shown in Table 2. The values of the multiplicity were obtained by reffering to ^1H *J* resolved 2D NMR spectra, when clear multiplicity was observed, such as 32-CH_3, 33-CH_3 and 6'-CH_3 *etc.* The ^1H-^1H connectivity map (^1H-COSY) for eurocidin E is shown in Fig. 2, marked with the connectivities for the regions of C-6 to C-10 and C-28 to C-33. Without references to further structural information the coupling sequences for the following parts of the molecule were able to be correctly interpreted: C-2 to C-4, C-6 to C-10, C-12 to C-18, C-29 to C-33 and mycosamine moiety. The cross peaks and chemical shifts of the regions of C-12 to C-18 and the mycosamine (C-1' to C-6') almost agreed with the data of amphotericins A and B[3]. Therefore it was estimated that the region of C-11 to C-18 contained a hemiketal (C-11) and C-14 (with a carboxyl group) had the same partial structure as amphotericins A and B, as well as mycosamine binding site at C-17.

Some polyenes such as nystatin A_1, pimaricin, tetrins A and B[5~7], as well as amphotericins A and B had the same partial structure as the region of C-11 to C-18 of eurocidin E. The common structures of such polyenes are suggested to be a mycosamine moiety, a hemiketal and a carboxyl group. The ^1H-COSY spectra of the region of C-2 to C-10 revealed that the cross peak connectivities were separated into two parts of the regions of C-2 to C-4 and C-6 to C-10. C-3 is considered to be a ketone carbon reffered to both chemical shifts of 2-H and 4-H protons and ^{13}C NMR data. Although it is possible that the carbons of C-2 to C-10 are sequenced in the reverse direction, the chemical shifts of 2-H (2.78 ppm) and 10-H protons (1.48 ppm) suggests that C-2 and C-10 are adjacent to the lactone and hemiketal carbons, respectively.

The comparison between eurocidins D and E in their ^1H-COSY spectra revealed that the cross assignments are identical with each other except for the region of C-6 to C-10. It is considered that one methylene-proton among C-6 to C-10 in eurocidin E had changes to a hydroxyl group in eurocidin D. ^1H NMR partial spectra (1.2~3.0 ppm) of eurocidins D and E are shown in Fig. 3. A doublet signal of two 8-H protons at 1.13 ppm in eurocidin E disappeared, and a doublet 8-H proton signal appeared at 2.98 ppm in eurocidin D (Fig. 3B and Table 2). The chemical shifts of methylene protons of 6-H, 7-H and 10-H of eurocidin D were not equivalent, and a 9-H proton signal was changed from broad singlet at 3.90 ppm in eurocidin E to a doublet at 3.80 ppm in eurocidin D. Therefore, the structure of eurocidin D

Table 2. ^{1}H NMR data of eurocidins D and E and their comparison with amphotericins A and B.

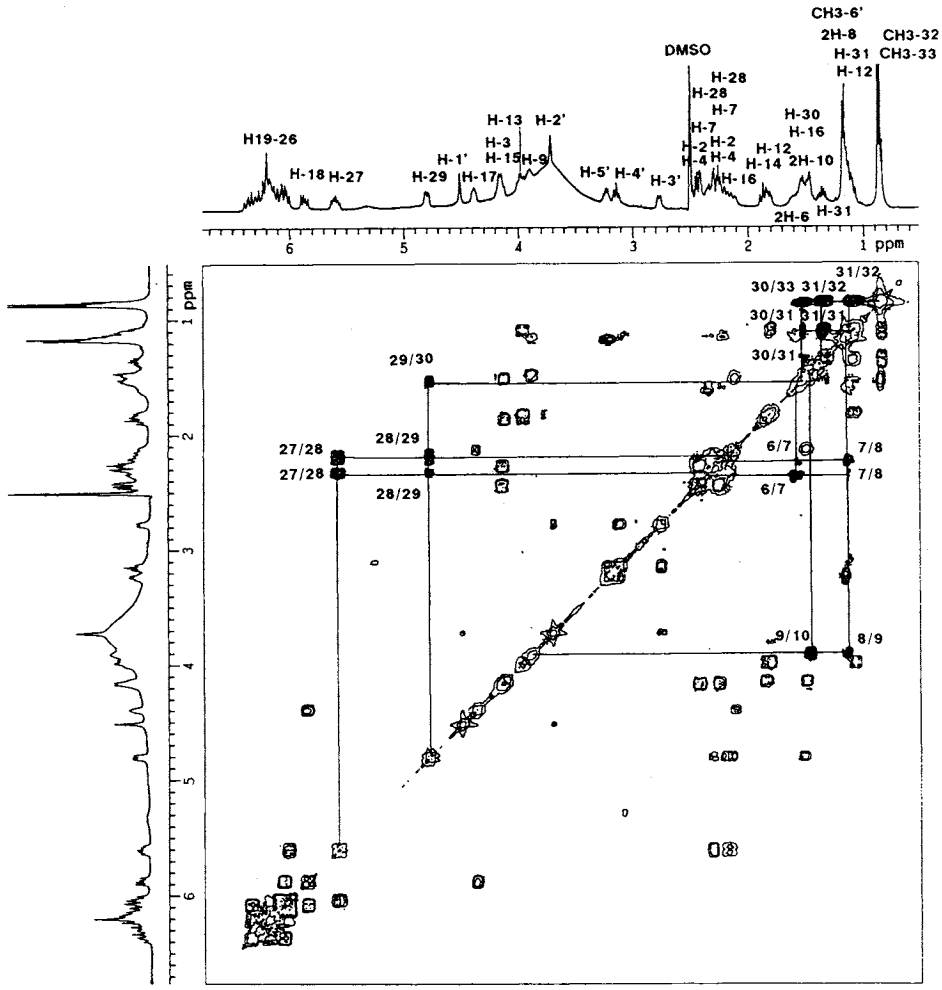
Proton[b]	Chemical shift (ppm), multiplicity (J(Hz))[a]			
	Eurocidin D	Eurocidin E	Amphotericin A[c]	Amphotericin B[c]
2-H	2.26	2.28 m	2.27	2.12
2-H	2.47	2.45 m	2.34	
3-H	4.17	4.16 m	4.03	4.07
4-H	2.26	2.28 m		
4-H	2.47	2.45 m		
6-H_2		1.60		
6-H	2.39			
6-H	2.57			
7-H	1.28	2.26		
7-H	1.66	2.42		
8-H_2		1.13 d (5.4)	1.50	
8-H	2.98 d (11.7)			
9-H	3.80 d (11.6)	3.90 br s	4.22	4.22
10-H_2		1.48 d (15.0)	1.60	
10-H	1.38 d (16.6)			
10-H	1.79			
12-H	1.10	1.10	1.13	1.10
12-H	1.82	1.81	1.88	1.80
13-H	3.99 m	3.98 m	3.95	3.96
14-H	1.87	1.87 dd (10.4, 10.3)	1.88	1.86
15-H	4.17	4.16 m	3.95	4.17
16-H	1.51	1.53	1.75	1.47
16-H	2.12	2.14	1.77	2.18
17-H	4.38 br s	4.39 br s	4.34	4.28
18-H	5.89 dd (15.6, 8.5)	5.87 dd (15.1, 8.4)	5.75	5.94
19-H~26-H	6.00~6.40	6.00~6.40		
27-H	5.61 m	5.60 m	5.69	
28-H	2.22	2.20 m		
	2.32	2.34		
29-H	4.79 dd (10.7, 4.1)	4.80 dd (10.2, 3.0)		
30-H	1.54	1.57 m		
31-H	1.10	1.10		
	1.35 ddd (13.2, 7.9, 5.2)	1.35 ddd (11.2, 7.7, 4.8)		
32-CH_3	0.85 t (7.3)	0.84 t (7.4)		
33-CH_3	0.86 d (6.8)	0.85 d (6.8)		
Mycosamine				
1′-H	4.47 s	4.51 s	4.47	4.46
2′-H	3.70 d (1.5)	3.72 d (2.8)	3.70	3.79
3′-H	2.74 d (8.8)	2.77 d (7.5)	2.68	2.96
4′-H	3.12	3.14	3.07	3.18
5′-H	3.20 m	3.23 m	3.14	3.24
6′-CH_3	1.17 d (6.0)	1.17 d (6.1)	1.13	1.15

[a] When clear multiplets were observed, they are indicated here.
[b] Two numbers at one carbon indicate that the two hydrogens on the same carbon are not equivalent.
[c] In literature[3].

is proposed that which a hydroxyl group of 8-position is substituted for a 8-H proton of eurocidin E as shown in Fig. 1.

Both eurocidins D and E are proposed to have novel pentaenic structures revealed at the first time among eurocidin family, and eurocidin D is proposed as a novel substance. On the other hand it was not found that eurocidin E was different from eurocidin A, a component of Eurocidin-T, from several

Fig. 2. Contour plot of a 2D correlated ^1H NMR spectrum and ^1H-^1H correlation map of eurocidin E (C-6 to C-10 and C-29 to C-33 regions).



HPLC analyses in the preceding paper[1] and some structural data[2], but the producing microorganisms of these two compounds and their productive patterns of eurocidin family were different from each other. Eurocidin E may be an isomer of eurocidin A such as a position isomer of hydroxyl groups or a stereoisomer. Whether eurocidin E is identical to eurocidin A remains still unknown until elucidating the structure and the properties of purified eurocidin A from Eurocidin-T.

The configuration of asymmmetric carbon atoms of eurocidins D and E is still unknown. The biological effects of eurocidins C, D and E on rat peritoneal mast cells compared with other polyenes, as well as their anti-microbial activities will be reported elsewhere[8].

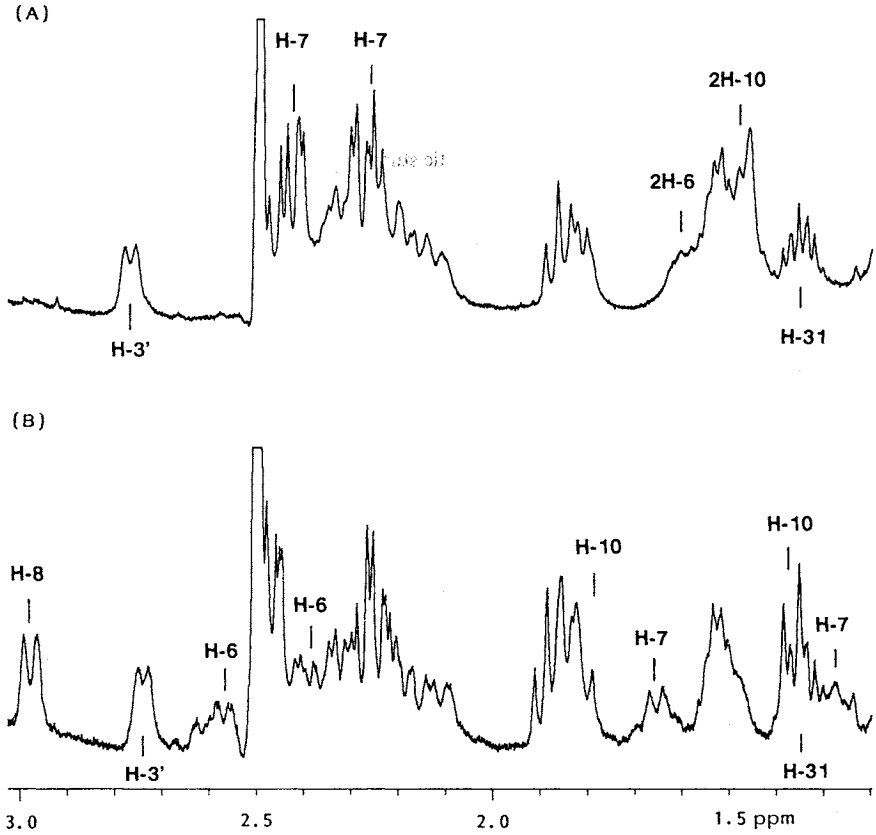
Experimental

Chemicals

Reference substance was a gift from Takeda Chemical Industries Ltd., Japan, designated as Eurocidin-T in this paper. DMSO-d_6 (99.95% purity) was purchased from E. Merck, Darmstadt, FRG.

Fig. 3. ^{1}H NMR partial spectra of eurocidins E and D.

(A) Eurocidin E, (B) eurocidin D.



NMR Spectrometry

NMR spectra were obtained on a Jeol JNM GX-400 spectrometer operating at 400 MHz with ^{1}H NMR and ^{1}H-^{1}H shift correlated 2D NMR (^{1}H-COSY). ^{13}C NMR, DEPT experiments (θ=45°, 90° and 135°) and ^{1}H J resolved 2D NMR were recorded with Jeol JNM GX-270 spectrometer operating at 270 MHz. The amounts of sample used were about 10 mg in 1.0 ml of DMSO-d_6 for ^{1}H experiments and about 25 mg in 0.7 ml of DMSO-d_6 for ^{13}C experiments. Chemical shifts were given in ppm using DMSO-d_6 as the internal standard.

^{1}H-COSY spectra were measured by the use of a 2D correlation sequence with a 90° mixing pulse. Data processing was carried out with the standard Jeol soft ware. An f2 spectral width of 2,500 Hz over 1,024 data points gave a digital resolution of 4.88 Hz. A total of 512 spectra, each of 32 transients, gave, with appropriate incrementing of the evolution delay, an f1 width of 2,500 Hz and a digital resolution of 4.9 Hz (with zero filling). The ^{1}H J resolved 2D spectra were obtained with the usual pulse sequence. The spectral widths were 1,170.1 Hz in f2 and 50.0 Hz in f1, giving a digital resolution of 1.14 and 0.1 Hz, respectively, and the data points were 2,048 × 512 matrix each of 64 transients.

Acknowledgments

The authors deeply thank Dr. TOSHIFUMI AKIZAWA, Cancer Institute, Japanese Foundation for Cancer Research, for his kind advice of this structure elucidation.

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