STEREOCHEMISTRY OF ENACYLOXINS 2.† STRUCTURE ELUCIDATION OF DECARBAMOYL ENACYLOXIN Ha AND IVa, NEW MEMBERS OF ENACYLOXIN ANTIBIOTICS FROM *Frateuria* sp. W-315‡

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Abstract

Two new members of enacyloxins (ENXs) were isolated from the culture extract of *Frateuria* sp. W-315. Spectrometric analyses revealed their structures as decarbamoyl enacyloxin IVa and IIa, the latter preferentially existed in the hemiacetal form. Formation of these compounds were catalyzed by enzymes produced by the fungus. From the coupling constant values of the hemiacetal part of dec ENX IIa, the (17'*R**, 18'*S**, 19'*R**) relative configuration of ENXs was also elucidated.

Introduction

In the preceding paper (1), we described the determination of the absolute structure of the cyclohexane part of ENXs to be (1*S*, 3*R*, 4*S*). Our continuing investigation on the chemistry of ENXs, we isolated two new analogs, decarbamoyl ENX IIa **1** and IVa **2** (dec ENX IIa and IVa) (2,3). Here the structure determination of these compounds are reported.

† Part 1. The preceding paper of this issue.

‡ This work was carried out under supervision of the late Dr. Takeyoshi Sugiyama, who passed away on September 5, 1999 at 53 years of age. This paper is dedicated to his memory.

Table 1. ^{1}H- and ^{13}C-NMR spectral data for dec ENX IIa **1** and dec ENX IVa **2** (CD_3OD).

	1		**2**	
Position	^{1}H (400 MHz)	^{13}C (100 MHz)	^{1}H	^{13}C
1-COOH	-	182.7	-	180.0
1	2.45, m	40.1	2.44, dddd (11.3, 11.3, 3.3, 3.3)	41.6
2	1.72, ddd (13.5, 11.6, 2.1) 2.13 dm (11.6)	33.3	1.72, ddd (14.6, 11.3, 2.1) 2.15, dm (14.6)	33.8
3	5.20, m	73.4	5.21, m	74.1
4	3.70, ddd (9.7, 5.8, 2.7)	71.0	3.71, ddd (9.4, 5.6, 2.7)	71.4
5	1.79, m	29.8	1.80, m	30.1
6	1.53, m 2.00, m	28.6	1.55, m 2.00, m	29.1
1'	-	168.7	-	169.0
2'	6.02, d, (15.0)	121.5	6.01, d, (15.0)	121.8
3'	7.42, dd (15.0, 11.0)	146.7	7.42, dd (15.0, 11.4)	146.9
4'	6.52, dd (15.0, 11.0)	127.4	6.52, dd (15.0, 11.4)	127.5
5'	6.75, d (15.0)	146.5	6.76, d (15.0)	146.8
6'	-	137.4	-	137.3
7'	6.41, br.d (9.8)	136.9	6.41, br.d (10.6)	137.5
8'	6.75, dd (14.6, 9.8)	131.5	6.75, dd (14.6, 10.6)	132.0
9'	6.71, dd (14.6, 9.7)	131.6	6.72, dd (14.6, 9.9)	131.6
10'	6.42, d (9.7)	128.4	6.43, d (9.9)	128.2
11'	-	141.0	-	142.3
12'	2.88, dq (9.7, 6.6)	47.8	2.87, dq (10.5, 7.0)	47.8
13'	4.16, dd (9.7, 1.1)	73.4	3.96, dd (10.5, 1.0)	72.5
14'	3.44, d (1.1)	73.7	3.36, dd (8.4, 1.0)	74.5
15'	-	100.4	3.91, ddd (10.2, 8.4, 2.2)	69.8
16'	1.52, dd (13.1, 11.6) 2.43, dd (13.1, 5.0)	40.8	1.46, ddd (14.4, 10.2, 2.3) 2.12, ddd (14.4, 10.3, 2.1)	40.7
17'	3.97, ddd (11.6, 9.7, 5.0)	76.9	4.45, ddd (10.3, 2.2)	68.0
18'	3.36, dd (9.8, 9.7)	67.5	3.74, dt (7.7, 2.2)	71.4
19'	4.33, dd (9.8, 6.9, 1.0)	73.4	4.27, dd (7.7, 7.0)	74.7
20'	5.52, ddt (15.4, 6.9, 1.2)	127.4	5.58, ddt (15.4, 7.0, 1.5)	130.4
21'	5.83, dtd (15.4, 6.8, 1.0)	137.8	5.81, dtd (15.3, 6.8, 1.1)	136.9
22'	2.09, qdd (7.5, 6.8, 1.2)	26.4	2.09, qd (7.5, 6.8)	26.7
23'	1.01, t (7.5)	13.8	1.02, t (7.5)	14.1
24'	1.96, br.s	12.6	1.97, br.s	12.9
25'	1.11, d (6.6)	16.1	1.13, d (7.0)	16.5

The values in parentheses are coupling constant (Hz).

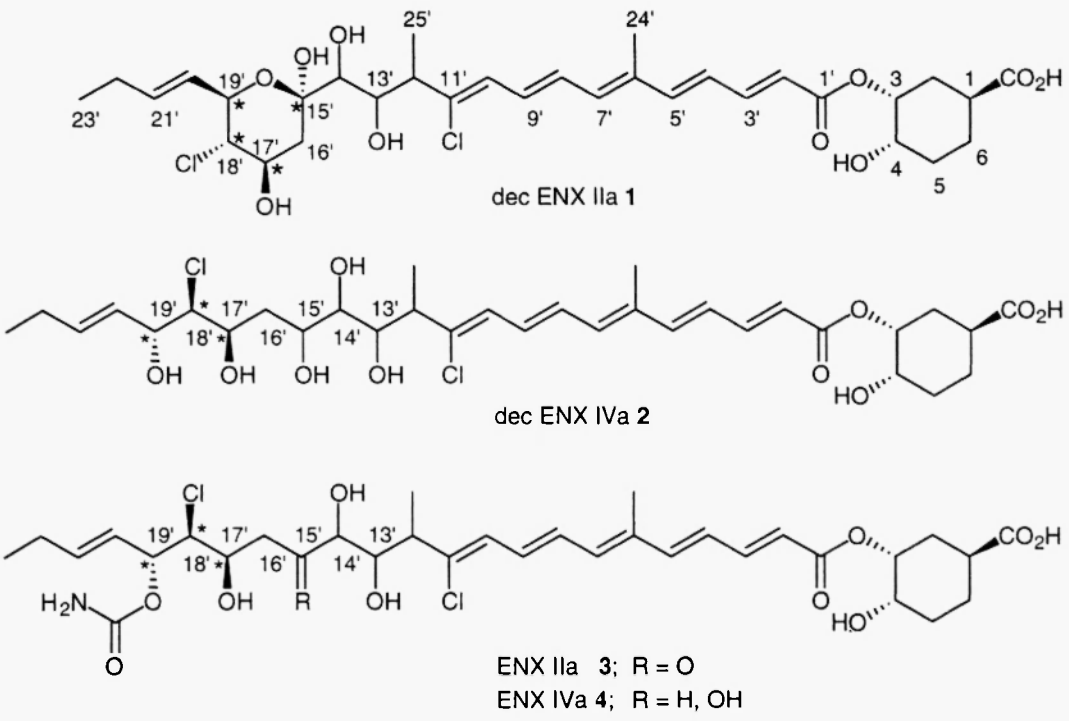

Fig. 1. Dec ENXs and ENXs.

Results and Discussion

In addition to ENX IIa **3** and IVa **4**, compounds **1** and **2** were isolated from the culture supernatant of *Frateuria* sp. W-315 (2,3) in 0.0001-0.001% yield as yellow oily substances. Both showed the quite similar UV and IR data (4). The molecular formula of compound **1** was established to be $C_{32}H_{44}Cl_2O_{10}$ by combined analyses of the HRFABMS (calcd. for M+Na$^+$, $C_{32}H_{44}Cl_2O_{10}Na$, 681.2209; found, 681.2233), and ^{1}H- and ^{13}C-NMR spectral data (Table 1). From the similarity of the NMR data, the absence of keto carbonyl function and the molecular formula, we concluded that **1** was a decarbamoylated derivative of **3**. This assumption was confirmed by the experimental results that **3** was converted into **1** when submitted to the culture supernatant (5), and **3** was recovered when the boiled culture supernatant was used (2). These results also showed that this transformation was catalyzed enzymatically. These behaviors were also observed for the compound **2** (5), which was revealed to be dec ENX IVa from combined analyses of the HRFABMS (calcd. for M+H$^+$, $C_{32}H_{47}Cl_2O_{10}$, 661.2654; found, 661.2601), and ^{1}H- and ^{13}C-NMR spectral data. As for the fact that ENX IIa **3** was enzymatically produced from ENX IVa **4**, dec ENX IIa **1** was also formed from dec ENX IVa **2** (3).

The relative configuration of the hemiacetal part was elucidated by the ^{1}H NMR coupling constant values. As shown in Fig.2, 1,2-diaxial relationships were observed from 16' to 19' protons. The hemiacetal hydroxy group would be axially oriented. Consequently, the relative configuration of the hemiacetal part of **1** was 15'*R**, 17'*R**, 18'*S**, 19'*R**. This configuration could be applied to the 17' to 19' position of other ENXs, such as ENX IIa **3** and ENX IV **4**.

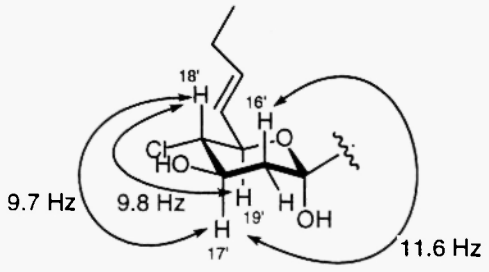

Fig. 2. The relative structure of the hemiacetal part of **1**.

Conclusion

New members of enacyloxin antibiotics lacking the carbamoyl group, dec ENX IIa **1** and dec ENX IVa **2**, were isolated from the culture extract of *Frateuria* sp. W-315. The configuration was determined to be 1*S*, 3*R*, 4*S*, 15'*R**, 17'*R**, 18'*S**, 19'*R**. Determination of the whole stereochemistry is under investigation.

Acknowledgement

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References and Notes

1 T. Fujimori, O. Nakayama, H. Kiyota, Y. Kamijima, T. Watanabe and T. Oritani, the preceding paper of this issue.

2 T. Watanabe, Ph. D. Thesis, Tohoku University, Japan, 1993.

3 a) R. Oyama, T. Watanabe, H. Hanzawa, T. Sano, T. Sugiyama and K. Izaki, Biosci. Biotechnol. Biochem., **58**, 1914 (1994). b) T. Watanabe, R. Oyama, H. Hanzawa, T. Sugiyama, K. Izaki, Biosci. Biotechnol. Biochem., **59**, 123 (1995).

4 IR (KBr) ν_{max} cm^{-1} 3420 (s, O-H), 1700 (s, C=O), 1620 (m), 1570 (m), 1245 (m), 1065 (m) and 970 (m); UV (MeOH) λ_{max} nm 268, 365 and 383.

5 Incuvation of these compounds in the culture supernatant revealed that dec ENX IVa **2** was directly converted into dec ENX IIa **1**; while ENX IIa **3** was once decarbamoylated to give dec ENX IIa in its linear (hydroxy ketone) form, then cyclyzed to form dec ENX IIa **1** (HPLC analysis (ODS 120A, MeCN/H_2O/HCO_2H = 1000:1200:6.6, A_{365}).

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