NEW POLYENIC ANTIBIOTICS ACTIVE AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

VII. ISOLATION AND STRUCTURE OF ENACYLOXIN IVa, A POSSIBLE BIOSYNTHETIC INTERMEDIATE OF ENACYLOXIN IIa

TOSHIHIKO WATANABE, JUN SHIMA, KAZUO IZAKI and TAKEYOSHI SUGIYAMA

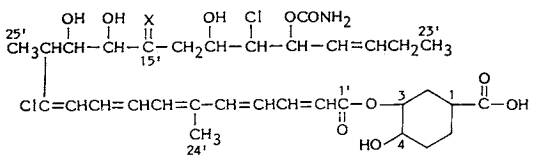
The Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai 981, Japan

(Received for publication September 4, 1991)

During the course of studies on the unique polyenic antibiotics produced by *Frateuria* (formerly *Gluconobacter*) sp. W-315, we isolated one of them, enacyloxin IIa (ENX IIa, **1**), and reported its structural elucidation[1,2]. In the fermentation of this bacterium, we found that the composition of the products varied depending on the culture phase. In the middle or late phase of fermentation the main product is **1**; whereas another compound, distinguished from **1** was produced in the early phase. This substance was eluted more rapidly than **1** in reversed-phase HPLC (CH_3CN-H_2O-HCOOH, 1,000:1,200:6.6) and its biological and physicochemical properties seemed to be quite similar to those of **1**. We isolated and purified this product according to our previous paper[3] and named it enacyloxin IVa (ENX IVa, **2**).

In this paper, we describe the isolation and chemical structure of **2**. The supernatant obtained by centrifugation of culture broth was extracted with ether after acidifying it with HCl to pH 2. The ether solution was then back-extracted with 1% $NaHCO_3$. An aqueous layer was acidified and re-extracted with ether. After evaporation of the solvent *in vacuo*, the residue was purified successively by preparative TLCs on Silica gel 60 (Merck, CH_2Cl_2-MeOH-AcOH, 65:5:1) and then on silanised Silica gel 60 (Merck, reversed-phase, CH_3CN-H_2O-AcOH, 40:60:0.4). The main band was scraped off from TLC plates, extracted with MeOH and concentrated *in vacuo*. Further purification was carried out by repeating the preparative TLC using both ordinary and reversed phases. During the purification, glassware and TLC plates were kept in darkness to protect the antibiotic from the light. One liter of culture yielded *ca.* 1 mg of ENX IVa (**2**).

Fig. 1. Structure of enacyloxin IIa (**1**) and enacyloxin IVa (**2**).



Enacyloxin IIa (**1**) X=O
Enacyloxin IVa (**2**) X=H, OH

The chemical structure of **2** was elucidated by spectroscopic analyses. IR and UV spectra of **2** were almost superimposable on those of **1**. It was suggested that **2** had functionalities similar to those of **1**, *i.e.* polyols, ester, carboxylic acid and polyene groups. The splitting pattern around $(M+Na)^+$ ion in the FAB-MS revealed the presence of two chlorine atoms in the molecule. Based on the HRFAB-MS data (726.2398 $(M+Na)^+$, calcd for $C_{33}H_{47}O_{11}NCl_2Na$=726.2423), the molecular formula of **2** was determined to be $C_{33}H_{47}O_{11}NCl_2$. The number of hydrogen atoms increased from 45 in **1** to 47 in **2**, meaning a less unsaturated structure for **2** compared to **1**. ^{13}C and ^1H NMR spectra of **2** were obtained in CD_3OD solution and the results are compared with those of **1** (Table 1). In ^{13}C NMR spectra, 33 signals were observed ($-CH_3\times3$, $>CH_2\times5$, $>CH-\times2$, $>CH-X$ (X=O or Cl)$\times8$, $-CH=\times10$, $>C=\times2$, $-COOR\times1$, $-COOH\times1$ and $-OCONH_2\times1$). The signal of ketone (211.7 ppm) in **1** disappeared in **2**, but eight oxymethines were observed in **2** instead of the seven in **1**. These observations allowed us to anticipate that **2** would be a structurally related congener of **1** with a reduced functionality at C-15′. The arrangement of carbon skeleton and functional groups was elucidated by detailed analysis of ^1H NMR spectra measured at 400 MHz. Phase-sensitive double quantum filter COSY spectra revealed the presence of spin networks of cyclohexane moiety, 2′-H~5′-H, 7′-H~10′-H and 12′-H~23′-H. As expected, methylene protons at 16′, 17′-H, 14′-H and 13′-H shifted to the higher magnetic fields and the new oxymethine proton of 15′-H was observed at 3.89 ppm. Other signals including olefinic ones were easily assigned from similarities to those of **1**.

In conclusion, the chemical structure of newly isolated antibiotic enacyloxin IVa was determined to be (2′*E*,4′*E*,6′*E*,8′*E*,10′*Z*,20′*E*)-3-(19′-carbamoyloxy-11′,18′-dichloro-6′,12′-dimethyl-13′,14′,15′,17′-tetrahydroxytricosa-2′,4′,6′,8′,10′,20′-hexaenoyl-

Table 1. ^{13}C- and ^{1}H-resonances of enacyloxins IIa (**1**) and IVa (**2**).

Position	Enacyloxin IIa (**1**)		Enacyloxin IVa (**2**)	
	^{13}C	^{1}H (J=Hz)	^{13}C	^{1}H (J=Hz)
1-COOH	181.5		181.0	
1	40.1	2.49 (dddd, J=11.6, 11.6, 3.5, 3.5)	39.0	2.54 (br t, J=10.9)
2	32.9	1.72 (ddd, J=14.1, 12, 3.0)	32.5	1.73 (ddd, J=14.5, 10.9, 2.4)
		2.15 (dm, J=14.2)		2.16 (m)
3	73.5	5.21 (dm, J=4.3)	73.2	5.20 (m)
4	70.8	3.72 (ddd, J=9.4, 6.4, 2.9)	70.6	3.73 (ddd, J=9.2, 5.9, 2.7)
5	29.7	1.80 (m)	29.5	1.80 (m)
6	28.2	1.55 (m), 2.00 (m)	27.7	1.57 (m), 2.04 (m)
1′	168.6		168.5	
2′	121.5	6.02 (d, J=15.1)	121.3	6.02 (d, J=15.0)
3′	146.7	7.42 (dd, J=15.0, 11.2)	146.8	7.43 (dd, J=15.0, 11.1)
4′	127.2	6.52 (dd, J=15.0, 11.2)	127.1	6.53 (dd, J=15.2, 11.1)
5′	146.5	6.75 (d, J=15.0)	146.6	6.76 (d, J=15.2)
6′	137.4		137.2	
7′	136.9	6.41 (br d, J=10.1)	137.0	6.42 (d, J=9.4)
8′	131.5	6.76 (dd, J=14.9, 10.1)	131.3	6.74 (dd, J=15.0, 9.4)
9′	131.6	6.71 (dd, J=14.9, 9.8)	131.8	6.72 (dd, J=15.0, 9.7)
10′	128.4	6.44 (d, J=9.8)	127.9	6.43 (d, J=9.7)
11′	140.6		142.0	
12′	47.5	2.94 (dq, J=9.5, 6.7)	47.5	2.87 (dq, J=9.7, 6.8)
13′	74.0	4.05 (br d, J=9.3)	72.3	3.94 (dd, J=9.7, 1.0)
14′	78.8	4.25 (br s)	74.2	3.35 (dd, J=8.0, 1.0)
15′	211.7		69.6	3.89 (ddd, J=10.6, 8.0, 2.2)
16′	44.6	2.84 (dd, J=17.0, 4.6)	40.4	1.47 (ddd, J=14.6, 10.6, 2.3)
		3.05 (dd, J=17.0, 7.9)		2.20 (ddd, J=14.6, 10.0, 2.3)
17′	66.9	4.50 (m)	69.6	4.23 (ddd, J=10.0, 2.3, 2.3)
18′	67.9	4.05 (dd, J=7.9, 2.5)	67.6	3.93 (dd, J=8.0, 2.3)
19′	75.5	5.28 (dd, J=7.8, 7.3)	75.8	5.29 (dd, J=8.0, 7.3)
19′-$OCONH_2$	158.6		158.8	
20′	126.1	5.55 (ddt, J=15.3, 7.3, 1.2)	126.2	5.56 (ddt, J=15.2, 7.3, 1.1)
21′	139.2	5.89 (dt, J=15.3, 6.4)	139.0	5.89 (dt, J=15.2, 6.5)
22′	26.3	2.10 (qdd, J=7.4, 6.4, 1.2)	26.3	2.10 (qdd, J=7.3, 6.5, 1.1)
23′	13.5	1.01 (t, J=7.4)	13.6	1.01 (t, J=7.3)
24′	12.7	1.95 (br s)	12.6	1.96 (s)
25′	16.2	1.19 (d, J=6.7)	16.2	1.12 (d, J=6.8)

oxy)-4-hydroxy-1-cyclohexanecarboxylic acid. It is noteworthy that, as mentioned above, **2** is the main product in the early phase of fermentation and **1** is that of the middle or late phase. Our unpublished data suggest that the amount of **2** in the early phase of culture decreased with the increase of **1** with the lapse of fermentation time and also suggest that these changes in the products seemed to be stoichiometric. It seemed likely therefore that, in the fermentation by *Frateuria* sp. W-315, **2** is probably the biosynthetic precursor to **1**.

As for biological activities, **2** was active against Gram-positive and Gram-negative bacteria, but inactive against yeasts and fungi. MIC against *E. coli* K-12 was shown to be 5.5 μg/ml. These properties were quite similar to those of **1**.

References

1) WATANABE, T.; T. SUGIYAMA, M. TAKAHASHI, J. SHIMA, K. YAMASHITA, K. IZAKI, K. FURIHATA & H. SETO: The structure of enacyloxin II, a novel linear polyenic antibiotic produced by *Gluconobacter* sp. W-315. Agric. Biol. Chem. 54: 259~261, 1990

2) WATANABE, T.; T. SUGIYAMA, M. TAKAHASHI, J. SHIMA, K. YAMASHITA, K. IZAKI, K. FURIHATA & H. SETO: New polyenic antibiotics active against Gram-positive and Gram-negative bacteria. IV. Structural elucidation of enacyloxin IIa. J. Antibiotics 45: 470~475, 1992

3) WATANABE, T.; K. IZAKI & H. TAKAHASHI: New polyenic antibiotics active against Gram-positive and -negative bacteria. I. Isolation and purification of antibiotics produced by *Gluconobacter* sp. W-315. J. Antibiotics 35: 1141~1147, 1982