CEPHABACINS, NEW CEPHEM ANTIBIOTICS OF BACTERIAL ORIGIN

II. ISOLATION AND CHARACTERIZATION

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Fifteen components of new antibiotics, cephabacins, were isolated from the culture filtrates of *Lysobacter lactamgenus* YK-90, *Xanthomonas lactamgena* YK-280 and *X. lactamgena* YK-278. They were purified by column chromatography using cation-exchange resins, activated carbon, high porous resins and cation-exchange Sephadex and by preparative reverse-phase HPLC. The basic, water-soluble antibiotics were characterized as having a cephem skeleton and oligopeptide(s) as a side chain constituent from their spectroscopic analyses and amino acid analyses.

A screening program for new β-lactam antibiotics, led to the discovery of three new bacterial species, *Lysobacter lactamgenus* YK-90, *Xanthomonas lactamgena* YK-280 and *X. lactamgena* YK-278[1], which produce 15 new cephem antibiotics having 7-formylaminodeacetylcephalosporin C or deacetylcephalosporin C as a skeleton and oligopeptide(s) as a side chain at the 3-position[2]. The compounds having a 7-formylamino group were named cephabacin $F_{1\sim9}$ and those having 7-hydrogen, cephabacin $H_{1\sim6}$. Cephabacins show broad antimicrobial activities *in vitro* and relatively strong protective effects *in vivo*[3]. Cephabacins belonging to the F group are very stable to all of the β-lactamases tested due to the presence of a formylamino group at the 7-position[3].

This paper deals with the isolation, physico-chemical properties and chemical characterization of cephabacins.

Isolation Procedure

The cephabacin isolation procedure is shown in Fig. 1.

These water-soluble, basic antibiotics were isolated by column chromatography using cation-exchange resins, high porous resins, cation-exchange Sephadex and activated carbon.

Gross separation into three groups was accomplished with Diaion HP-20 chromatography. Each fraction was successively separated into three components by CM-Sephadex chromatography. In the final stage, each crude component was purified by preparative reverse-phase HPLC.

The active fractions were detected by antimicrobial activity using strains hypersensitive to β-lactam antibiotics *Pseudomonas aeruginosa* C_{88} and *Escherichia coli* PG-8, and by HPLC. Components $F_{1\sim3}$ and $H_{1\sim3}$, $F_{4\sim6}$ and $H_{4\sim6}$, and $F_{4\sim9}$ and $H_{4\sim6}$ were obtained from the culture filtrates of *L. lactamgenus* YK-90, *X. lactamgena* YK-280 and *X. lactamgena* YK-278, respectively. Deacetylcephalosporin C was isolated from the cells of *X. lactamgena* YK-280 and YK-278.

Physico-chemical Properties

Amphoteric substances of cephabacin group antibiotics were isolated as white powders of hydro-

Fig. 1. Isolation procedure of cephabacins.

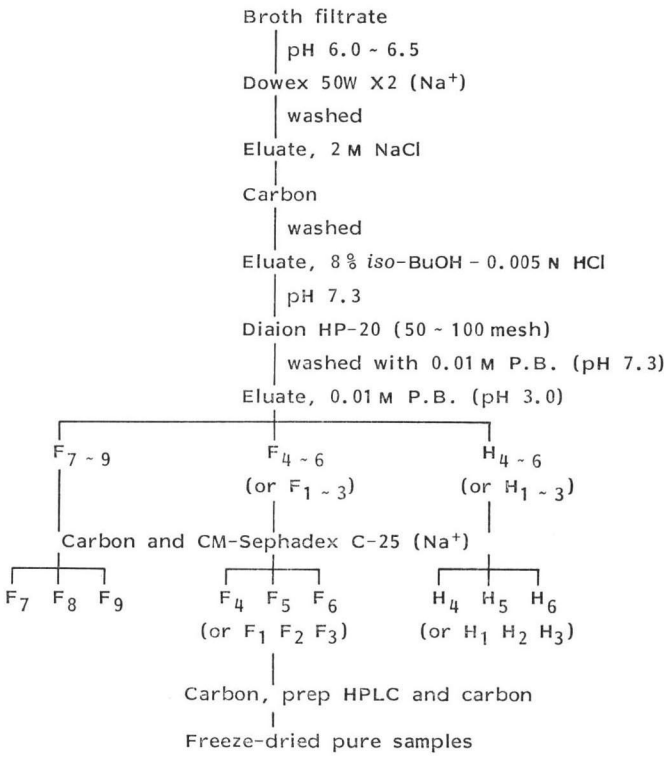


Table 1. Mobilities of cephabacins on TLC and HPLC.

TLC

Component	Rf	Component	Rf	Component	Rf	Component	Rf	Component	Rf
F_1	0.52	F_4	0.58	F_7	0.45	H_1	0.50	H_4	0.62
F_2	0.55	F_5	0.61	F_8	0.47	H_2	0.54	H_5	0.64
F_3	0.60	F_6	0.68	F_9	0.51	H_3	0.58	H_6	0.67

Adsorbent; cellulose, Spot film (Tokyo Kasei).
Solvent system; CH_3CN - 3% $(NH_4)_2SO_4$ (4: 1).
Detection; UV lamp at 254 nm, bioautography using *E. coli* PG-8.

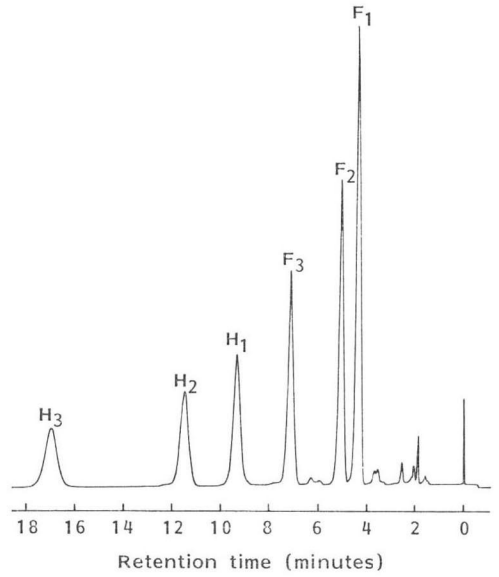
HPLC

Component	Rt	Component	Rt	Component	Rt	Component	Rt	Component	Rt
F_1	5.8	F_4	3.7	F_7	1.9	H_1	12.2	H_4	7.9
F_2	6.8	F_5	4.2	F_8	2.2	H_2	15.2	H_5	9.6
F_3	11.7	F_6	4.6	F_9	2.4	H_3	22.5	H_6	10.1

Column; ODS, YMC Pack A-323 (Yamamura Chem. Lab.).
Equipment; Model 6000A/660/440 (Waters Ass.).
Mobile phase; 2% MeOH - 0.01 M P.B. (pH 3.0).
Flow rate; 2 ml/minute, Rt values are expressed in minute.
Detection; UV absorbance at 254 nm.

chlorides. They give positive color reactions for ninhydrin, Greig-Leaback and Sakaguchi (excluding $F_{7\sim9}$) reagents and negative color reactions for potassium permanganate and Barton reagents. The antibiotics are easily soluble in water and sparingly soluble in dimethyl sulfoxide, methanol or acetone. The chromatographic mobilities on TLC and HPLC are shown in Table 1. The typical HPLC pattern of cephabacin $F_{1\sim3}$ and $H_{1\sim3}$ is shown in Fig. 2.

Fig. 2. HPLC pattern of cephabacin $F_{1\sim3}$ and $H_{1\sim3}$.
Column: YMC Pack A-312 ODS.
Mobile phase: 2% MeOH - 0.01 M P.B. (pH 3.0).



The stabilities of cephabacin $F_{1\sim3}$ and H_1 at 60°C in phosphate buffer (P. B.) solutions of various pH are shown in Table 2. Cephabacins are stable in acidic and neutral pH regions and relatively unstable in the basic pH region. These stability data show that cephabacins are slightly unstable in comparison with cephalosporin C.

The physico-chemical properties and spectral data of cephabacin components are shown in Table 3. The specific rotations of cephabacins indicated larger values with a decrease in molecular weight in each group. The molecular formulae were determined from elemental analyses, molecular ion peaks in secondary ion mass spectrometry (SI-MS) and carbon numbers in ^{13}C NMR spectrometry. The UV spectra of cephabacins showed maxima at 260 nm ($E^{1\%}_{1cm}$ 91~124). The CD spectra had two contrary Cotton effects at 226~228 nm (negative) and at 256~258 (positive). The IR spectra indicated absorptions at 1775~1780 (β-lactam for F components) or 1765~1770 (β-lactam for H components), 1730~1735 (ester) and 1660~1675 (amide) cm^{-1}. These spectral data strongly suggested that cephabacins have the same cephem skeleton. Typical UV, CD and IR spectra of cephabacins are shown in Figs. 3, 4 and 5.

Chemical Characterization

Cephabacins were assumed to have cephalosporin and alanine or serine moieties from their ^1H

Table 2. Stabilities of cephabacins in aqueous solutions.

Component	Half-life time (hours)			
	pH 3	pH 5	pH 7	pH 9
F_1	1.4	0.73	0.87	0.50
F_2	0.87	0.87	1.0	0.50
F_3	1.3	1.0	1.0	0.50
H_1	0.85	0.85	0.85	0.58
Cephalosporin C	1.6	1.5	1.5	0.90
7-Formylaminodeacetylcephalosporin C	1.6	19.7	19.7	3.6
Deacetylcephalosporin C	1.9	27.4	29.0	6.3

Concentration of samples; 100 μg/ml in 0.01 M P. B.
Temperature; 60°C.
Detection; HPLC method.

Table 3. Physico-chemical properties of cephabacins.

Component	$[\alpha]_D^{25}$ in H_2O (c)	SI-MS[a] $(M+H)^+$	*Anal* Found: upper column, Calcd: lower column						Molecular formula (Hydrochloride)	UV $\lambda_{max}^{H_2O}$ nm ($E_{1cm}^{1\%}$)	CD $(\theta)_{(nm)}^{H_2O}$	IR ν_{max}^{KBr} cm^{-1} β-CO ester amide
			C	H	N	O	S	Cl				
F_1	+71.8°	688	38.29	6.48	15.11		4.12	8.71	$C_{26}H_{41}N_9O_{11}S$	260 (117)	−30,900 (228)	1775, 1730, 1670
	(0.50)		38.33	6.06	15.47	27.49	3.94	8.70	$\cdot 2HCl(3H_2O)$		+29,500 (260)	
F_2	+54.8°	759	39.02	6.51	15.46		3.50	8.27	$C_{29}H_{46}N_{10}O_{12}S$	260 (113)	−33,600 (228)	1780, 1735, 1675
	(0.56)		39.32	6.14	15.81	27.09	3.62	8.01	$\cdot 2HCl(3H_2O)$		+30,700 (260)	
F_3	+25.1°	830	34.61	6.54	15.92		3.41	6.41	$C_{32}H_{51}N_{11}O_{13}S$	260 (106)	−34,700 (228)	1780, 1735, 1665
	(0.49)		40.17	6.22	16.10	26.75	3.35	7.41	$\cdot 2HCl(3H_2O)$		+28,400 (260)	
F_4	+74.1°	704	38.49	6.03	15.63		4.22	7.95	$C_{26}H_{41}N_9O_{12}S$	260 (124)	−43,000 (226)	1780, 1730, 1670
	(0.59)		38.43	5.83	15.51	27.56	3.95	8.73	$\cdot 2HCl(2H_2O)$		+31,000 (258)	
F_5	+58.8°	791	40.00	5.83	15.64		4.30	4.53	$C_{29}H_{46}N_{10}O_{14}S$	260 (108)	−31,000 (226)	1780, 1730, 1660
	(0.57)		40.35	5.95	16.23	29.65	3.71	4.11	$\cdot HCl(2H_2O)$		+29,000 (258)	
F_6	+35.0°	862	38.04	6.30	14.30		3.18	7.76	$C_{32}H_{51}N_{11}O_{15}S$	260 (97)	−28,000 (226)	1780, 1735, 1660
	(0.52)		38.17	6.11	15.30	30.19	3.18	7.04	$\cdot 2HCl(4H_2O)$		+26,000 (258)	
F_7	+63.0°	676	40.38	6.53	12.81		4.07	8.40	$C_{26}H_{41}N_7O_{12}S$	260 (118)	−34,000 (226)	1780, 1735, 1675
	(0.50)		39.79	6.04	12.50	28.54	4.09	9.04	$\cdot 2HCl(2H_2O)$		+27,000 (258)	
F_8	+56.1°	763	39.34	6.02	12.52		4.40	7.57	$C_{29}H_{46}N_8O_{14}S$	260 (124)	−39,000 (226)	1780, 1735, 1670
	(0.54)		39.95	6.01	12.85	29.38	3.68	8.13	$\cdot 2HCl(2H_2O)$		+29,000 (258)	
F_9	+47.9°	834	39.66	6.44	12.84		3.35	7.62	$C_{32}H_{51}N_9O_{15}S$	260 (104)	−50,700 (226)	1780, 1740, 1680
	(0.50)		40.00	6.19	13.12	29.97	3.34	7.38	$\cdot 2HCl(3H_2O)$		+41,200 (258)	
H_1	+53.5°	645	40.30	6.44	15.34		4.39	4.66	$C_{25}H_{40}N_8O_{10}S$	260 (122)	−50,700 (226)	1765, 1735, 1665
	(0.51)		40.84	6.44	15.24	28.29	4.36	4.82	$\cdot HCl(3H_2O)$		+21,000 (258)	
H_2	+31.1°	716	39.86	6.28	14.64		3.79	7.83	$C_{28}H_{45}N_9O_{11}S$	260 (114)	−41,200 (226)	1770, 1735, 1660
	(0.51)		39.90	6.34	14.96	26.58	3.80	8.41	$\cdot 2HCl(3H_2O)$		+21,000 (256)	
H_3	+5.8°	787	40.27	6.52	14.52		2.92	7.12	$C_{31}H_{50}N_{10}O_{12}S$	260 (94)	−30,000 (226)	1770, 1735, 1660
	(0.49)		40.74	6.40	15.33	26.26	3.51	7.76	$\cdot 2HCl(3H_2O)$		+17,000 (258)	
H_4	+45.0°	661	37.51	6.28	14.10		4.00	9.94	$C_{25}H_{40}N_8O_{11}S$	260 (112)	−45,700 (226)	1770, 1735, 1670
	(0.54)		38.12	6.14	14.23	28.44	4.07	9.00	$\cdot 2HCl(3H_2O)$		+22,800 (258)	
H_5	+34.5°	748	39.71	5.87	14.85		3.90	7.46	$C_{28}H_{45}N_9O_{13}S$	260 (109)	−49,800 (226)	1765, 1730, 1660
	(0.58)		40.10	5.89	15.03	26.71	3.82	8.45	$\cdot 2HCl(H_2O)$		+26,300 (256)	
H_6	+20.2°	819	38.00	6.87	14.35		3.10	7.78	$C_{31}H_{50}N_{10}O_{14}S$	260 (91)	−43,000 (226)	1770, 1735, 1660
	(0.50)		38.63	6.27	14.53	29.88	3.33	7.36	$\cdot 2HCl(4H_2O)$		+28,000 (256)	

[a] Hitachi M-80A, xenon ion beam source, glycerin matrix.

Fig. 3. UV spectrum of cephabacin F_1.

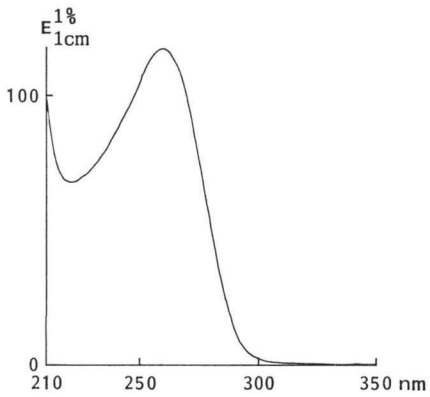


Fig. 4. CD spectrum of cephabacin F_1.

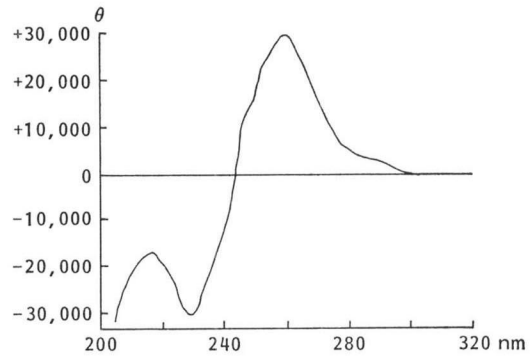
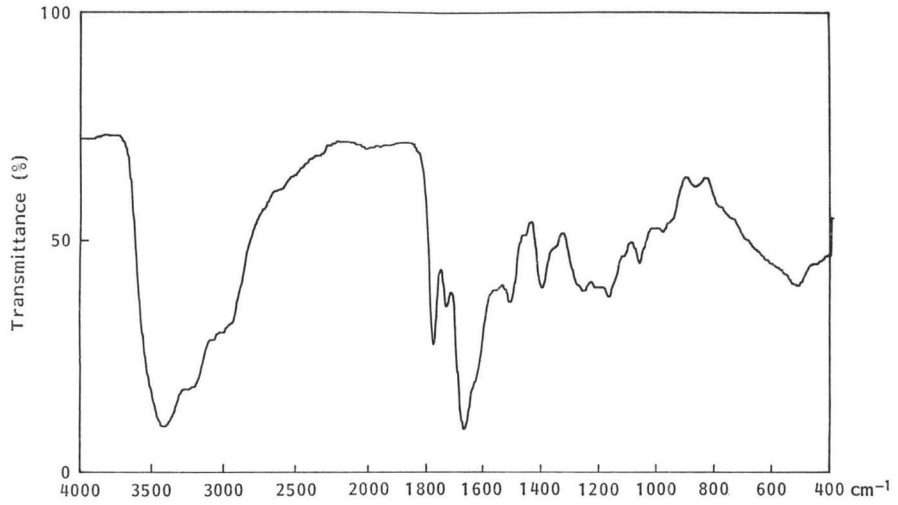


Fig. 5. IR spectrum of cephabacin F_1.



NMR (Table 4) and ^{13}C NMR (Table 5) spectra. The numbering for the assignment of the NMR signals is shown in Fig. 6. Amino acid analyses of the hydrolysates in 5.5 N HCl gave the data shown in Table 6. Alanine was detected in the components, $F_{1\sim3,8,9}$ and $H_{1\sim3,8}$ and serine in $F_{4\sim9}$ and $H_{4\sim8}$. α-Aminoadipic acid was also detected in all samples. About one-third to half equimolar glycine was detected in the components belonging to the cephabacin F group as very close peaks with those of α-aminoadipic acid. The cleavage mechanism for glycine of this type has been reported in the chemistry of A16886 B[4] (cephamycin C)[5]. The absolute configurations of alanine, serine and α-aminoadipic acid were determined to be of the L-, L- and D-form, respectively, by the HPLC method described in the literature[6]. The presence of L-alanine and L-serine in each component was confirmed by the difference from the molecular ion peaks in the SI-MS and by addition of the signals in the ^{13}C NMR spectra (Tables 3 and 5), for example, among F_1, F_2 and F_3 or H_1, H_2 and H_3.

The existence of a formyl group in the F components was clarified with the signals at δ 8.16 ppm (s) in the ^1H NMR spectra, the signals at 166.32~166.45 ppm (d) in the ^{13}C NMR spectra and subtraction of ion peaks in the SI-MS in comparison with the data of the corresponding components,

Table 4. ^{1}H NMR spectra of cephabacins ($\delta^{D_2O}_{ppm}$ J(Hz), 400 MHz, Jeol GX-400).

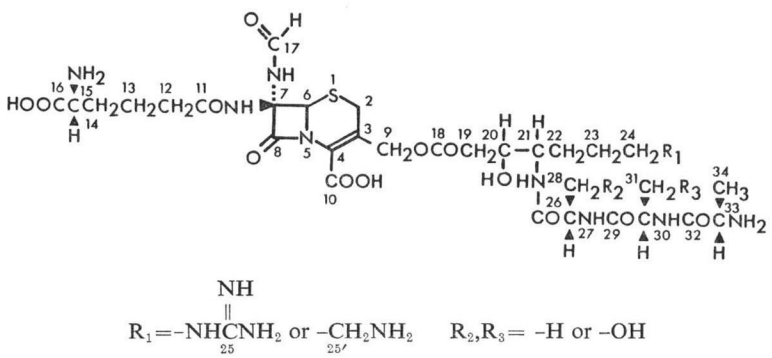
Position	F_1	F_2	F_3	F_4
2-H	3.31 d, 18.0	3.31 d, 17.5	3.31 d, 18.0	3.31 d, 17.8
	3.66 d, 18.0	3.66 d, 17.5	3.66 d, 18.0	3.66 d, 17.8
6-H	5.34 s	5.34 s	5.34 s	5.34 s
7-H				
9-H	4.74 d, 12.5	4.71 d, 12.5	4.71 d, 12.5	4.71 d, 12.9
	4.89 d, 12.5	4.88 d, 12.5	4.88 d, 12.5	4.92 d, 12.9
12-H	2.44 t, 7.0	2.45 t, 7.0	2.45 t, 7.0	2.44 t, 7.1
13-H	1.57~1.84 m	1.60~1.86 m	1.60~1.87 m	1.60~1.84 m
14-H	1.84~2.04 m	1.86~2.02 m	1.87~2.02 m	1.84~2.05 m
15-H	3.79 t, 6.0	3.74 t, 6.0	3.74 t, 6.0	3.85 t, 6.0
17-H	8.16 s	8.16 s	8.16 s	8.16 s
19-H	2.51 dd, 9.0, 16.0	2.48 dd, 9.5, 15.5	2.46 dd, 9.5, 15.5	2.50 dd, 9.4, 15.6
	2.65 dd, 4.0, 16.0	2.65 dd, 3.5, 15.5	2.65 dd, 3.5, 15.5	2.68 dd, 3.7, 15.6
20-H	4.05 m	4.00 m	3.98 m	4.05 m
21-H	3.88 m	3.80 m	3.80 m	3.91 m
22-H	1.38~1.57 m	1.36~1.60 m	1.36~1.60 m	1.38~1.60 m
	1.57~1.84 m	1.60~1.86 m	1.60~1.87 m	1.60~1.84 m
23-H	1.38~1.57 m	1.36~1.60 m	1.36~1.60 m	1.38~1.60 m
	1.57~1.84 m	1.60~1.86 m	1.60~1.87 m	1.60~1.84 m
24-H	3.20 t, 6.5	3.20 t, 6.5	3.19 t, 6.5	3.20 t, 6.6
25′-H				
27-H	4.07 q, 7.0	4.09 q, 7.0	4.09 q, 7.0	4.13 dd, 4.2, 5.6
28-H	1.54 d, 7.0	1.53 d, 7.0	1.53 d, 7.0	3.94 dd, 5.6, 12.5
				4.01 dd, 4.2, 12.5
30-H		4.29 q, 7.0	4.22 q, 7.0	
31-H		1.41 d, 7.0	1.40 d, 7.0	
33-H			4.33 q, 7.0	
34-H			1.39 d, 7.0	

Position	F_7	H_1	H_2	H_3
2-H	3.33 d, 17.8	3.40 d, 18.0	3.40 d, 17.7	3.40 d, 17.8
	3.66 d, 17.8	3.67 d, 18.0	3.68 d, 17.7	3.66 d, 17.8
6-H	5.34 s	5.13 d, 4.6	5.13 d, 4.5	5.12 d, 4.6
7-H		5.64 d, 4.6	5.65 d, 4.5	5.64 d, 4.6
9-H	4.77 d, 12.7	4.74 d, 12.4	4.74 d, 12.5	4.74 d, 12.5
	4.93 d, 12.7	4.96 d, 12.4	4.97 d, 12.5	4.98 d, 12.5
12-H	2.44 t, 7.1	2.42 t, 7.2	2.43 t, 7.5	2.42 t, 7.3
13-H	1.58~1.84 m	1.60~1.82 m	1.60~1.84 m	1.60~1.85 m
14-H	1.84~2.04 m	1.82~2.00 m	1.84~1.98 m	1.85~2.00 m
15-H	3.84 t, 6.2	3.74 t, 6.1	3.75 t, 6.1	3.81 t, 6.0
17-H	8.16 s			
19-H	2.49 dd, 9.5, 15.5	2.53 dd, 9.0, 15.6	2.49 dd, 9.4, 15.6	2.47 dd, 9.5, 15.4
	2.68 dd, 3.5, 15.5	2.67 dd, 4.0, 15.6	2.66 dd, 3.9, 15.6	2.65 dd, 3.5, 15.4
20-H	4.06 ddd, 3.5, 5.5, 9.5	4.08 m	4.01 m	3.98 m
21-H	3.90 m	3.89 m	3.83 m	3.80 m
22-H	1.38~1.54 m	1.42~1.60 m	1.44~1.60 m	1.36~1.60 m
	1.58~1.84 m	1.60~1.82 m	1.60~1.84 m	1.60~1.85 m
23-H	1.26~1.38 m	1.42~1.60 m	1.44~1.60 m	1.36~1.60 m
	1.38~1.54 m	1.60~1.82 m	1.60~1.84 m	1.60~1.85 m
24-H	1.58~1.84 m	3.21 t, 6.6	3.21 t, 6.6	3.19 t, 6.8
25′-H	2.98 t, 7.3			
27-H	4.12 dd, 4.1, 5.7	4.08 q, 7.1	4.09 q, 7.3	4.09 q, 7.1
28-H	3.94 dd, 5.7, 12.3	1.55 d, 7.1	1.54 d, 7.3	1.53 d, 7.1
	4.00 dd, 4.1, 12.3			
30-H			4.31 q, 7.3	4.22 q, 7.1
31-H			1.42 d, 7.3	1.40 d, 7.1
33-H				4.32 q, 7.1
34-H				1.38 d, 7.1

Table 5. ^{13}C NMR spectra of cephabacins

Position	F_1	F_2	F_3	F_4	F_5	F_6	F_7
11-C	179.84 (s)	179.78 (s)	179.79 (s)	179.79 (s)	179.85 (s)	179.52 (s)	179.74 (s)
16-C	177.42 (s)	177.92 (s)	178.04 (s)	177.00 (s)	177.20 (s)	178.64 (s)	177.19 (s)
18-C	176.05 (s)	176.15 (s)	176.12 (s)	176.11 (s)	176.24 (s)	176.16 (s)	176.16 (s)
26-C	173.75 (s)	173.49 (s)	173.47 (s)	170.93 (s)	171.13 (s)	173.94 (s)	170.90 (s)
29-C		176.88 (s)	177.38 (s)		174.41 (s)	174.49 (s)	
32-C			177.47 (s)			175.71 (s)	
10-C	171.12 (s)	170.77 (s)	171.08 (s)	170.77 (s)	171.13 (s)	169.48 (s)	170.66 (s)
17-C	166.40 (d)	166.41 (d)	166.32 (d)	166.42 (d)	166.42 (d)	166.45 (d)	166.42 (d)
8-C	162.16 (s)	162.25 (s)	162.07 (s)	162.24 (s)	162.23 (s)	162.54 (s)	162.12 (s)
25-C	159.62 (s)	159.59 (s)	159.52 (s)	159.63 (s)	159.61 (s)	159.67 (s)	
4-C	134.86 (s)	134.39 (s)	135.00 (s)	134.46 (s)	134.55 (s)	132.57 (s)	134.89 (s)
3-C	117.40 (s)	118.69 (s)	117.27 (s)	118.40 (s)	117.97 (s)	123.59 (s)	117.77 (s)
7-C	79.64 (s)	79.66 (s)	79.57 (s)	79.67 (s)	79.67 (s)	79.79 (s)	79.70 (s)
20-C	72.66 (d)	72.91 (d)	72.86 (d)	72.70 (d)	72.86 (d)	72.33 (d)	72.71 (d)
9-C	67.16 (t)	67.11 (t)	67.10 (t)	67.07 (t)	67.10 (t)	66.82 (t)	67.12 (t)
6-C	65.94 (d)	66.04 (d)	65.88 (d)	66.01 (d)	66.00 (d)	66.47 (d)	66.02 (d)
15-C	57.34 (d)	56.95 (d)	57.28 (d)	57.03 (d)	57.18 (d)	56.26 (d)	57.35 (d)
21-C	56.31 (d)	56.03 (d)	55.87 (d)	56.52 (d)	56.30 (d)	56.21 (d)	56.67 (d)
27-C	52.03 (d)	51.72 (d)	51.62 (d)	57.58 (d)	57.40 (d)	58.28 (d)	57.58 (d)
30-C		53.14 (d)	53.07 (d)		59.10 (d)	58.97 (d)	
33-C			52.35 (d)			51.93 (d)	
24-C	43.59 (t)	43.61 (t)	43.52 (t)	43.61 (t)	43.58 (t)	43.62 (t)	31.28 (t)
25′-C							42.22 (t)
19-C	41.23 (t)	41.52 (t)	41.52 (t)	41.18 (t)	41.46 (t)	41.46 (t)	41.21 (t)
12-C	37.36 (t)	37.32 (t)	37.27 (t)	37.35 (t)	37.36 (t)	37.25 (t)	37.36 (t)
14-C	32.80 (t)	32.62 (t)	32.73 (t)	32.68 (t)	32.74 (t)	32.33 (t)	32.77 (t)
22-C	28.98 (t)	29.23 (t)	29.25 (t)	28.98 (t)	29.33 (t)	29.33 (t)	29.30 (t)
2-C	28.60 (t)	28.66 (t)	28.48 (t)	28.65 (t)	28.66 (t)	28.91 (t)	28.62 (t)
23-C	27.50 (t)	27.50 (t)	27.36 (t)	27.52 (t)	27.41 (t)	27.35 (t)	25.08 (t)
13-C	23.50 (t)	23.47 (t)	23.41 (t)	23.50 (t)	23.52 (t)	23.41 (t)	23.50 (t)
28-C	19.75 (q)	19.55 (q)	19.45 (q)	63.26 (t)	63.19 (t)	63.87 (t)	63.22 (t)
31-C		19.51 (q)	19.42 (q)		63.89 (t)	64.06 (t)	
34-C			19.25 (q)			19.47 (q)	

Fig. 6. Numbering for the assignment of the NMR signals.



for example, between F_1 and H_1, F_2 and H_2, or F_3 and H_3. The guanidyl group also existed in the components, $F_{1\sim6}$ and $H_{1\sim6}$, according to the positive Sakaguchi reactions and the signals at δ 159.54~159.69 ppm (s) in the ^{13}C NMR spectra when compared with those of the $F_{7\sim9}$ components.

($\delta^{D_2O}_{ppm}$, 100 MHz, Jeol GX-400 at 4~7°C).

F_8	F_9	H_1	H_2	H_3	H_4	H_5	H_6
179.69 (s)	179.68 (s)	179.52 (s)	179.48 (s)	179.39 (s)	179.36 (s)	179.41 (s)	179.38 (s)
177.19 (s)	177.18 (s)	177.43 (s)	177.82 (s)	177.87 (s)	176.10 (s)	176.44 (s)	177.10 (s)
176.21 (s)	176.24 (s)	176.05 (s)	176.12 (s)	176.15 (s)	175.86 (s)	176.20 (s)	176.14 (s)
171.04 (s)	173.90 (s)	173.72 (s)	173.46 (s)	173.45 (s)	170.69 (s)	171.02 (s)	173.90 (s)
174.14 (s)	174.36 (s)		177.36 (s)	177.26 (s)		174.23 (s)	174.16 (s)
	174.44 (s)			176.47 (s)			174.34 (s)
170.89 (s)	170.81 (s)	171.61 (s)	171.49 (s)	170.68 (s)	170.18 (s)	170.84 (s)	171.22 (s)
166.38 (d)	166.37 (d)						
162.07 (s)	162.03 (s)	167.89 (s)	167.83 (s)	168.00 (s)	168.18 (s)	168.01 (s)	167.70 (s)
		159.57 (s)	159.54 (s)	159.65 (s)	159.69 (s)	159.66 (s)	159.61 (s)
134.95 (s)	135.06 (s)	134.40 (s)	134.37 (s)	133.51 (s)	132.59 (s)	133.50 (s)	134.57 (s)
117.55 (s)	117.40 (s)	118.99 (s)	118.99 (s)	121.03 (s)	122.91 (s)	121.05 (s)	118.85 (s)
79.68 (s)	79.67 (s)	61.99 (d)	61.95 (d)	62.04 (d)	62.08 (d)	62.06 (d)	61.99 (d)
72.84 (d)	72.87 (d)	72.64 (d)	72.84 (d)	72.90 (d)	72.69 (d)	72.83 (d)	72.73 (d)
67.09 (t)	67.14 (t)	67.23 (t)	67.19 (t)	67.15 (t)	67.04 (t)	67.11 (t)	67.17 (t)
66.00 (d)	66.02 (d)	60.13 (d)	60.10 (d)	60.21 (d)	60.24 (d)	60.21 (d)	60.10 (d)
57.36 (d)	57.41 (d)	57.32 (d)	57.31 (d)	56.84 (d)	56.57 (d)	56.82 (d)	57.40 (d)
56.37 (d)	56.28 (d)	56.28 (d)	55.96 (d)	55.91 (d)	56.39 (d)	56.27 (d)	56.36 (d)
57.41 (d)	58.21 (d)	51.97 (d)	51.63 (d)	51.77 (d)	57.58 (d)	57.38 (d)	58.23 (d)
59.00 (d)	58.94 (d)		53.04 (d)	53.02 (d)		58.99 (d)	58.86 (d)
	51.91 (d)			52.44 (d)			51.89 (d)
31.52 (t)	31.52 (t)	43.53 (t)	43.53 (t)	43.64 (t)	43.64 (t)	43.62 (t)	43.56 (t)
42.25 (t)	42.23 (t)						
41.41 (t)	41.49 (t)	41.18 (t)	41.47 (t)	41.54 (t)	41.19 (t)	41.40 (t)	41.41 (t)
37.35 (t)	37.32 (t)	37.58 (t)	37.53 (t)	37.47 (t)	37.41 (t)	37.49 (t)	37.49 (t)
32.77 (t)	32.76 (t)	32.75 (t)	32.72 (t)	32.54 (t)	32.36 (t)	32.54 (t)	32.74 (t)
29.20 (t)	29.18 (t)	28.94 (t)	29.14 (t)	29.30 (t)	28.97 (t)	29.23 (t)	29.20 (t)
28.60 (t)	28.56 (t)	28.43 (t)	28.38 (t)	28.56 (t)	28.71 (t)	28.59 (t)	28.37 (t)
24.94 (t)	24.86 (t)	27.46 (t)	27.43 (t)	27.41 (t)	27.51 (t)	27.40 (t)	27.27 (t)
23.49 (t)	23.46 (t)	23.85 (t)	23.81 (t)	23.77 (t)	23.71 (t)	23.78 (t)	23.79 (t)
63.10 (t)	63.84 (t)	19.70 (q)	19.44 (q)	19.55 (q)	63.21 (t)	63.12 (t)	63.81 (t)
63.90 (t)	64.03 (t)		19.44 (q)	19.44 (q)		63.92 (t)	63.90 (t)
	19.41 (q)			19.37 (q)			19.38 (q)

Discussion

Flavobacterium sp. SC 12,154 has recently been reported as a producer of deacetoxycephalosporin C in the cell[7], and 7-formamidocephalosporins have been isolated as acetyl derivatives (SQ 28,516 and 28,517) from the filtrate[8]. However, we have isolated 15 components of cephabacins from bacterial broths without derivatization. They showed unique biological activities[3].

As cephabacins showed mobilities similar to labile basic peptides in the primary stage of isolation, the purification processes were carried out below 7°C. But they were not as unstable in various pH solutions as some carbapenems, which are labile at acidic pH ranges[9,10]. Cephabacins are unstable due to the 3-ester bond as 7-formylaminodeacetylcephalosporin C and oligopeptides[2] are very stable in aqueous solutions. The difference of stability between cephabacins and 7-formylaminodeacetylcephalosporin C corresponds to that between cephalosporin C and deacetylcephalosporin C (Table 2).

Cephabacin $F_{1\sim3}$ and $H_{1\sim3}$ were isolated on the same level from the filtrate of the flask culture but cephabacin $F_{1\sim3}$ were the major products in large-scale fermentation. This tendency was also observed in the case of cephabacin $F_{4\sim6}$ and $H_{4\sim6}$, suggesting that fermentation control should enable the production of a specific target component.

Our findings show that cephabacin is unambiguously a new family of cephem antibiotics binding to oligopeptides. Their unique structures are discussed in the following paper.

Table 6. Amino acid analysis of cephabacin hydrolysates.

Component	Alanine	Serine	α-Aminoadipic acid	Glycine
F_1	0.86	—	0.94	0.42
F_2	1.9	—	1.0	0.46
F_3	3.1	—	1.1	0.51
F_4	—	0.90	1.6[a]	—
F_5	—	1.8	1.6[a]	—
F_6	0.90	1.8	1.6[a]	—
F_7	—	0.86	1.2	0.25
F_8	—	1.7	1.4	0.29
F_9	0.96	1.8	1.3	0.35
H_1	0.86	—	1.3[b]	—
H_2	1.9	—	1.3[b]	—
H_3	2.8	—	1.2[b]	—
H_4	—	0.95	1.3[b]	—
H_5	—	2.0	1.3[b]	—
H_6	0.94	2.0	1.3[b]	—

Conditions of hydrolysis; 5.5 N HCl, 110°C, 11~15 hours.
Equipment: Hitachi Amino Acid Autoanalyzer, Model 835-50.
[a] A glycine peak was overlapped to an α-aminoadipic acid peak.
[b] A small amount of glycine was detected.

Experimental

Isolation of Cephabacins

The culture broth of *L. lactamgenus* YK-90 (3,900 liters) was filtered with Hyflo-Super Cel after adjustment to pH 6.1. The filtrate (4,370 liters) cooled below 5°C was loaded onto Dowex 50 W (Na^+ type, 50~100 mesh, 120 liters) and antimicrobially active substances were eluted with 2 M NaCl (1,800 liters). The eluate was applied to activated carbon (60 liters) and active components were eluted with 8% 2-BuOH - 1/200 N HCl (420 liters). The concentrate (40 liters), after adjustment to pH 7.3, was chromatographed on Diaion HP-20 (40 liters) with 0.01 M P.B. (pH 3.5, 400 liters). The eluate was desalted with activated carbon (10 liters). The concentrate (2 liters) of the desalted eluate was again loaded onto Diaion HP-20 (50~100 mesh, 4 liters). The components were separated into two groups at this stage by the elution using 0.01 M P.B. (pH 3.5). The early eluate mainly containing $F_{1\sim3}$ was desalted with activated carbon (1.5 liters). The recovered antibiotics were applied to CM-Sephadex C-25 (Na^+ type, 1.5 liters) and eluted with 0.02 M NaCl (60 liters). The pure fractions detected by HPLC were individually combined and desalted with activated carbon to give freeze-dried F_1 (7.5 g), F_2 (10.8 g) and F_3 (2.7 g).

The later eluate mainly containing $H_{1\sim3}$ was desalted with activated carbon (0.3 liter). The recovered antibiotics were applied to CM-Sephadex C-25 (Na^+ type, 0.3 liter) and eluted with 0.02 M NaCl (12 liters). The fractions were analyzed by HPLC to divide three groups, mainly containing H_1, H_2 and H_3. The divided fractions were individually desalted with activated carbon and purified with preparative HPLC using TSK-GEL LS-410 (Toyo Soda). The pure fractions detected by HPLC were combined and desalted with activated carbon to give freeze-dried H_1 (30 mg), H_2 (63 mg) and H_3 (69 mg).

The culture broths of *X. lactamgena* YK-280 and YK-278 were treated with almost the same procedure. The yields are as follows;

Producing strain	Filtrate (liters)	Component	(mg)	Producing strain	Filtrate (liters)	Component	(mg)
YK-280	1,000	F_4	1,500			H_6	46
		F_5	2,000				
		F_6	1,900	YK-278	16	F_7	3
		H_4	332			F_8	8
		H_5	501			F_9	22

Isolation of Deacetylcephalosporin C

The culture broth of *X. lactamgena* YK-280[1] was centrifuged at 8,000 rpm for 20 minutes and the resulting cell was washed with water. This process was repeated twice. Washed cell was extracted with 70% Me_2CO (1.5 liters). The concentrate of the extract (150 ml) was loaded on activated carbon (10 ml) and active fractions were eluted with 50% Me_2CO (50 ml). The concentrate of the eluate (25 ml, pH 5.0) was applied to a column of IRA-68 (AcO^- type, 5 ml) and eluted with 0.5 M AcONa (50 ml, pH 3.9). The eluate was desalted with activated carbon (5 ml) and lyophilized. The crude powder (22.3 mg) was purified with QAE-Sephadex A-25 (Cl^- type, 5 ml) and eluted with 0.05 M NaCl. The active fractions were loaded on preparative HPLC using μ-BondaPak TM/C_{18} with elution of MeOH - 0.01 M P. B. (pH 3.0) (1: 99). Amount of deacetylcephalosporin C was estimated as 60 μg from the peak of the standard sample. Deacetylcephalosporin C obtained thus was identified by TLC, HPLC, UV and CD spectra and an antimicrobial spectrum.

The whole broth of *X. lactamgena* YK-278[1] (5 liters) was also treated with the same scale and procedure to give 60 μg of deacetylcephalosporin C.

Addendum from The Editorial Office

Cephabacins F_1 and F_2 are identical with chitinobolins A and B. (SHOJI, J., *et al.*: J. Antibiotics 37: 1486~1490, 1984)

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