CEPHABACINS, NEW CEPHEM ANTIBIOTICS OF BACTERIAL ORIGIN

III. STRUCTURAL DETERMINATION

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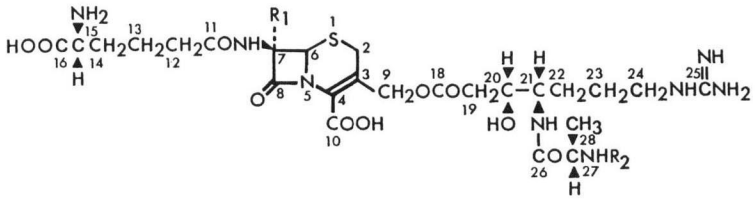
The structures of 15 new cephem antibiotics, cephabacin $F_{1\sim9}$ and $H_{1\sim6}$, were determined by their spectroscopic analyses and decomposition studies. They are consisted of a cephalosporin nucleus and a di, tri or tetrapeptide including a new amino acid which is bound at the position 3 with an ester bond. The components, $F_{1\sim9}$, showed unique biological activities by the presence of a formylamino group at the position 7.

The preceding paper[1] reported the isolation of 15 components of cephabacin $F_{1\sim9}$ and $H_{1\sim6}$ from culture filtrates of *Lysobacter lactamgenus* YK-90, *Xanthomonas lactamgena* YK-280 and *X. lactamgena* YK-278.[2] They show broad antimicrobial activities *in vitro*, remarkable stability to various types of β-lactamases and relatively strong protecting effects in experimentally infected mice.[3] 7-Formamidocephalosporins isolated as acetyl derivatives (SQ 28,516 and 28,517) from the culture filtrate of *Flavobacterium* sp. SC 12,154 have recently been reported.[4] This report deals with the structural determination of these new cephem antibiotics, cephabacins.

The preceding paper reported that cephabacins are composed of a cephalosporin nucleus and oligopeptide(s) based on their properties, spectral analysis data and amino acid analysis data.[1] Fig. 1 shows the reaction pathways and acidic decomposition studies. Cephabacin F_1 (**1**) gave a diacetylamino derivative (**2**), SI-MS *m/z* 794 (M+H), by Ac_2O in dimethylacetamide. Compound **2** shows a positive Sakaguchi reaction and a negative ninhydrin reaction. Cephabacin F_3 (**3**) also gave a dibenzoylamino derivative (**4**), SI-MS *m/z* 1,038 (M+H), by benzoyl chloride in 2% $NaHCO_3$. The *pKa'* values at 6.9 ($-NH_3^+$) and 8.5 ($-NH_3^+$) in **3** were absent in **4** and three *pKa'* values were observed at 3.1 ($-COO^-$), 3.9 ($-COO^-$) and >11 ($-NHC(=NH)NH_3^+$). These data indicate that cephabacin F_3 has a guanidyl, two amino and two carboxyl groups in the molecule as free hydrophilic functions.

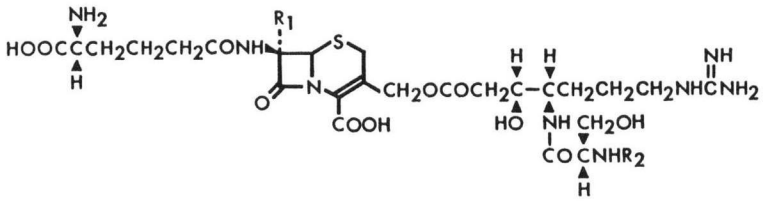
When the ^{13}C NMR signals attributed to a cephem skeleton, D-α-aminoadipic acid and L-alanine are subtracted from those of **1** (Table 2), the residual carbon number is nine including the carbon of a formyl group. Refluxing of **1** in 5.5 N HCl afforded a mixture of L-alanine, glycine and D-α-aminoadipic acid[1] and a mixture of three compounds originating from a new amino acid including a guanidyl group. One of the latter was 4-keto-7-guanidinoheptanoic acid (**5**), SI-MS *m/z* 202 (M+H), IR 1730 cm^{-1} (isolated CO). The other two compounds were benzoylated as a mixture and separated by preparative HPLC, giving 3-hydroxy-4-benzoylamino-7-guanidinoheptanoic acid (**6**), $[\alpha]_D^{25}$ $-31.0°$ (*c* 0.50, H_2O), SI-MS *m/z* 323 (M+H), and 4-benzoylamino-7-guanidino-2-heptenoic acid (**7**), SI-MS *m/z* 305 (M+H). The ^1H NMR signals of **6** in DMSO-d_6 showed similar patterns to the corresponding signals in **2** (Table 3). But the methylene and methyne signals at δ 2.00 (dd, J=9.3, 14.7 Hz), 2.21 (dd, J=2.6, 14.7 Hz) and 3.75 (m) ppm in **6** were not observed in the spectrum of **7** in DMSO-d_6,

Table 1. Structure of cephabacins.



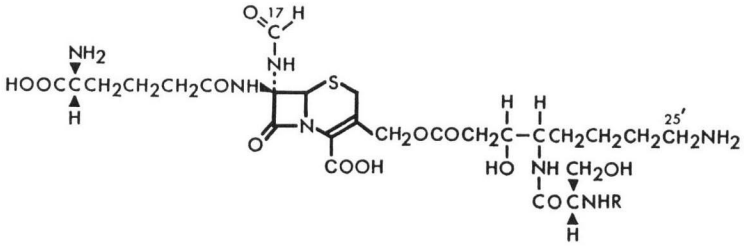
	R_1	R_2
F_1	-NHC(=O)H	-H
F_2	〃	←L-Ala
F_3	〃	←L-Ala←L-Ala

	R_1	R_2
H_1	-H	-H
H_2	〃	←L-Ala
H_3	〃	←L-Ala←L-Ala



	R_1	R_2
F_4	-NHC(=O)H	-H
F_5	〃	←L-Ser
F_6	〃	←L-Ser←L-Ala

	R_1	R_2
H_4	-H	-H
H_5	〃	←L-Ser
H_6	〃	←L-Ser←L-Ala



	R
F_7	-H
F_8	←L-Ser
F_9	←L-Ser←L-Ala

and new coupled signals at 5.73 (dd, J=1.5, 15.4 Hz) and 6.44 (dd, J=4.5, 15.4 Hz) were observed in **7**. Another methyne signal at δ 3.80 ppm (m) in **6** indicated a downfield shift to 4.52 (m) in **7** which was coupled with the signal at 6.44. In the ^{1}H NMR spectrum of **5**, five methylene signals at δ 1.85, 2.43, 2.64, 2.75 and 3.19 ppm were clearly observed and there was no methyne proton. These spectral data confirmed the structures of **5**, **6** and **7**. The debenzoyl compound of **6** was dehydrated at either side of the hydroxyl group by acid hydrolysis to form a double bond or an enamine (and followed by deamination).

Fig. 1.

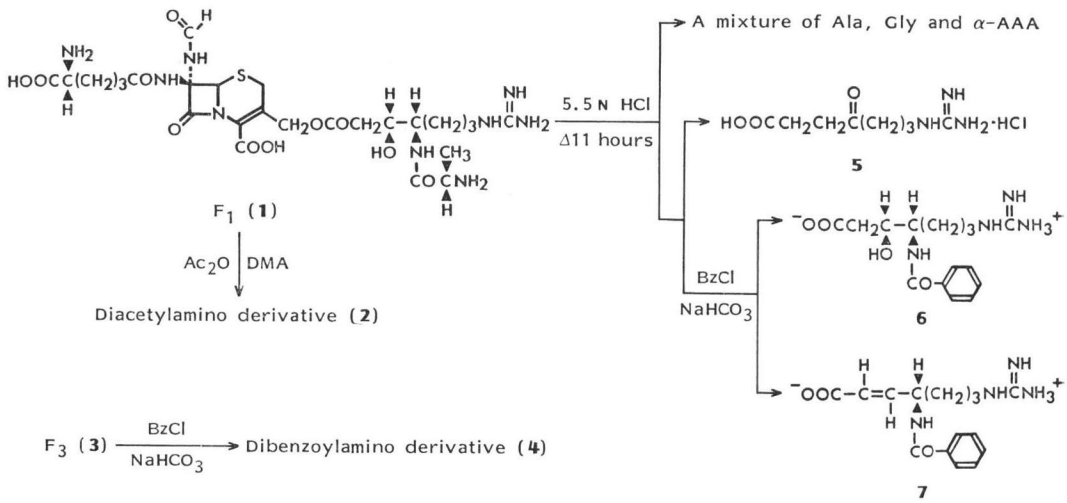


Table 2. ^{13}C NMR spectra ($\delta^{D_2O}_{ppm}$, 100 MHz, Jeol GX-400).

Position	1	8	9	11[a]	12	17	20
2-C	28.60 t	28.22 t		27.8 t			
3-C	117.40 s	122.50 s		123.6 s			
4-C	134.86 s	132.76 s		131.8 s			
6-C	65.94 d	66.03 d		59.6 d			
7-C	79.64 s	79.47 s		61.3 d			
8-C	162.16 s	162.01 s		167.1 s			
9-C	67.16 t	63.75 t		63.3 t			
10-C	171.12 s	171.43 s		171.1 s			
11-C	179.84 s	179.65 s		178.6 s			
12-C	37.36 t	37.28 t		37.0 t			
13-C	23.50 t	23.40 t		23.4 t			
14-C	32.80 t	32.70 t		32.3 t			
15-C	57.34 d	57.33 d		56.8 d			
16-C	177.42 s	177.22 s		167.5 s			
17-C	166.40 d	166.30 d					
18-C	176.05 s		182.31 s		181.22 s	182.26 s	182.36 s
19-C	41.22 t		43.75 t		43.47 t	43.73 t	43.70 t
20-C	72.66 d		73.71 d		70.84 d	73.69 d	73.73 d
21-C	56.31 d		56.53 d		57.87 d	56.76 d	56.81 d
22-C	28.98 t		29.02 t		27.27 t	29.10 t	29.17 t
23-C	27.50 t		27.46 t		27.17 t	27.47 t	24.98 t
24-C	43.59 t		43.58 t		42.53 t	43.61 t	31.33 t
25-C	159.62 s		159.59 s		159.67 s	159.62 s	
25′-C							42.16 t
26-C	173.75 s		173.53 s			170.62 s	171.20 s
27-C	52.03 d		52.01 d			57.59 d	57.71 d
28-C	19.75 q		19.57 q			63.19 t	63.49 t

[a] Varian XL-100 (25 MHz).

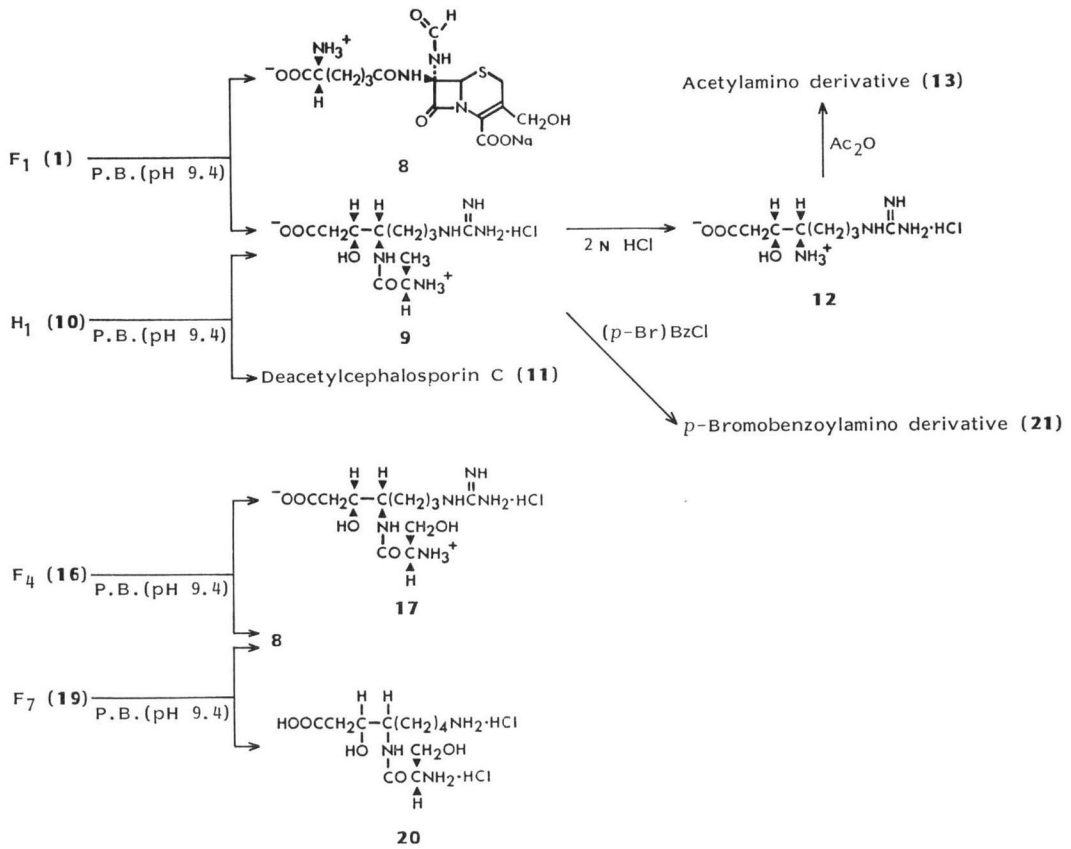
Mild alkaline hydrolysis gave clear-cut results for the determination of the structures as shown in Fig. 2. When **1** was hydrolyzed in phosphate buffer (P.B.) adjusted to pH 9.4 with NaOH, we obtained the sodium salt of 7-formylaminodeacetylcephalosporin C (**8**), $[\alpha]_D^{25}$ +150° (*c* 0.55, H_2O),

Table 3. ^{1}H NMR spectra ($\delta^{D_2O}_{ppm}$ J (Hz), 400 MHz, Jeol GX-400).

Position	2	8	9	11	12	17	20
2-H	3.33 d, 18.0	3.38 d, 17.6		3.46 d, 17.8			
		3.65 d, 18.0	3.65 d, 17.6		3.67 d, 17.8		
6-H	5.36 s	5.35 s		5.13 d, 4.6			
7-H				5.63 d, 4.6			
9-H	4.72 d, 12.5	4.21 d, 12.9	4.25 d, 12.9	4.26 d, 12.9	4.30 d, 12.9		
		4.88 d, 12.5					
12-H	2.40 m	2.46 t, 7.3		2.43 t, 7.1			
13-H	1.60~1.76 m	1.65~1.84 m		1.62~1.82 m			
14-H	1.76~1.92 m	1.84~2.04 m		1.82~2.00 m			
15-H	4.16 dd, 4.5, 7.5	3.75 dd, 5.5, 6.7		3.75 t, 6.0			
17-H	8.16 s	8.17 s					
19-H	2.48 dd, 9.5, 15.0		2.30 dd, 8.5, 15.1		2.41 dd, 7.8, 15.1	2.32 dd, 8.6, 15.1	2.30 dd, 8.6, 14.9
	2.67 dd, 3.5, 15.0		1.42 dd, 4.4, 15.1		2.46 dd, 5.6, 15.1	2.45 dd, 4.4, 15.1	2.44 dd, 4.6, 14.9
20-H	4.02 m		3.99 ddd, 4.4, 5.5, 8.5		4.26 ddd, 3.4, 5.6, 7.8	4.00 m	3.99 m, (19-Ha, b)
21-H	3.83 m		3.88 ddd, 3.2, 5.5, 9.5, (22-Ha, b)[a]		3.35 m	3.91 m	3.92 m, (22-Ha, b)
22-H	1.40~1.60 m		1.40~1.62 m, (21-H)		1.60~1.71 m	1.42~1.60 m	1.42~1.54 m, (21-H, 22-Hb)
	1.60~1.76 m		1.73~1.83 m, (21-H)		1.71~1.83 m	1.73~1.83 m	1.62~1.80 m, (21-H, 22-Ha)
23-H	1.40~1.60 m		1.40~1.62 m, (24-H)		1.60~1.71 m	1.42~1.60 m	1.30~1.42 m
	1.60~1.76 m		1.62~1.73 m, (24-H)		1.71~1.83 m	1.60~1.73 m	1.42~1.54 m
24-H	3.18 dt, 6.5, 13.5		3.21 t, 6.7, (23-Ha, b)		3.26 t, 5.9	3.21 t, 6.7	1.62~1.80 m, (25′-H, 23-Ha, b)
	3.22 dt, 6.5, 13.5						
25′-H							3.00 m, (24-H)
27-H	4.20 q, 7.0		4.08 q, 7.1			4.12 dd, 4.1, 5.6	4.09 t, 4.8, (28-H)
28-H	1.37 d, 7.0		1.56 d, 7.1, (27-H)			3.97 dd, 5.6, 12.5	3.97 m
	2.01 s, Ac					4.02 dd, 4.1, 12.5	
	2.04 s, Ac						

[a] The proton number in parentheses indicates the decoupled signal when the signal at this position was irradiated.

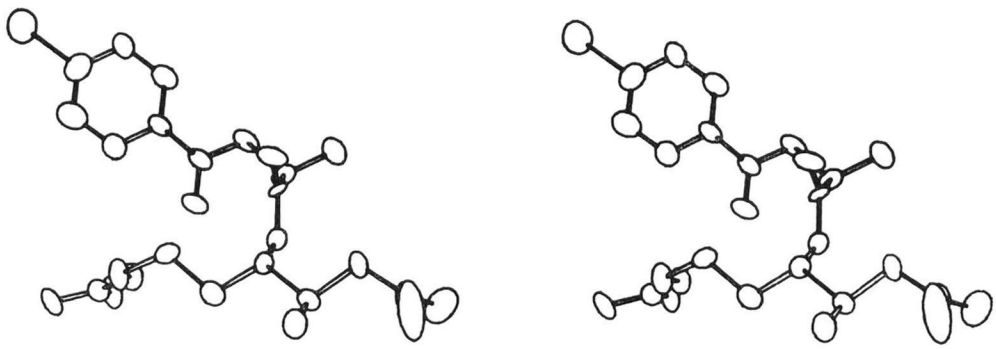
Fig. 2.



SI-MS m/z 439 (M+H), and 3-hydroxy-4-L-alanylamino-7-guanidinoheptanoic acid (**9**), $[\alpha]_D^{25}$ −16.4° (c 0.56, H_2O), SI-MS m/z 290 (M+H). In the same procedure, cephabacin H_1 (**10**) afforded deacetylcephalosporin C[5,6] (**11**), $[\alpha]_D^{25}$ +106.9° (c 0.5, H_2O), SI-MS m/z 396 (M+H), and **9**. The UV and CD spectra of **8** and **11** exhibited very similar maxima and Cotton effects at 258 nm (ε 9,500), $[\theta]_{228}$ −35,000, $[\theta]_{260}$ +32,000 and at 258 nm (ε 8,600), $[\theta]_{226}$ −33,000, $[\theta]_{258}$ +23,000, respectively. The SI-MS and NMR spectra showed that the structure of **8** differs from that of **11** only by a formylamino group. In the ^{13}C NMR spectra of **8** and **11** (Table 2), three signals from the β-lactam ring showed downfield shifts to δ 66.03 ppm (d, 6-C) from 59.6 (d) and to 79.47 (s, 7-C) from 61.3 (d) and an upfield shift to 162.01 (s, 8-C) from 167.1 (s). Other signals indicated almost the same chemical shifts and splitting patterns. These data strongly suggest that the formylamino group is attached at the 7-position. Selective proton decoupling studies by low power in **1** confirmed this assignment. When the signal of formyl proton at δ 8.16 ppm was irradiated, the 7-C signal at 79.64 ($^3J_{C-H}$=7.3 Hz) was decoupled to a singlet. The absolute configuration at the 7-position can be reasonably designated as R from the CD spectral data, but the large shifts of the Cotton effects on the methoxy group[7] in A-16886 B[8] (cephamycin C) were not observed.

Compound **9** was hydrolyzed by refluxing in 2 N HCl for 4 hours, giving L-alanine and a new amino acid, 3-hydroxy-4-amino-7-guanidinoheptanoic acid (**12**), $[\alpha]_D^{25}$ −6.3° (c 0.63, H_2O), SI-MS m/z 219 (M+H). The structure of **12** was confirmed by proton-spin-decoupling studies. When the methyne

Fig. 3. Stereoscopic view of the molecule **21**.



signal at δ 4.26 ppm (ddd) was irradiated, the methylene signals at 2.41 (dd, $J_{19a,b}$=15.1, $J_{19a,20}$=7.8) and 2.46 (dd, $J_{19b,20}$=5.6) and the methyne signal at 3.35 (m, $J_{20,21}$=3.4) collapsed two doublet and triplet-like signals, respectively. On the contrary, by irradiation of the methyne signal at δ 3.35 ppm, the methyne signal at 4.26 and the methylene signals at 1.60~1.71 (m) and 1.71~1.83 (m) collapsed the double doublet and sharpened the multiplet signals, respectively. By acetylation in Ac_2O and 3% $NaHCO_3$, **12** yielded acetylamino derivative **13**, SI-MS *m/z* 261 (M+H). These findings showed that L-alanine binds to the amino group of **12** at an acid amide bond.

The ^{13}C NMR spectrum of **1** indicated that it consists of compounds **8** and **9**. The DNP method for determination of the *N*-terminal yielded the dinitrophenyl derivatives of L-alanine and D-α-aminoadipic acid from **1**. The carboxyl group in **9** apparently combines with the 3-hydroxymethyl group in **8** by an ester bond which is consistent with the absorption at 1730 cm^{-1} in the IR spectrum of **1**.

Thus, the structure of **1** was determined to be 3-[4-L-alanylamino-7-guanidino-3-hydroxy]heptanoyl-7-formylaminodeacetylcephalosporin C.

The complex of cephabacin $F_{1\sim3}$ was hydrolyzed under mild basic condition and gave a tripeptide **14**, $[\alpha]_D^{25}$ −34.5° (*c* 0.53, H_2O), SI-MS *m/z* 361 (M+H), and a tetrapeptide **15**, $[\alpha]_D^{25}$ −60.2° (*c* 0.51, H_2O), SI-MS *m/z* 432 (M+H), together with **8** and **9**. Similar hydrolysis of pure samples of $F_{2\sim3}$ and $H_{1\sim3}$ gave the corresponding hydrolysates. The structures of cephabacin $F_{2\sim3}$ and $H_{1\sim3}$ were thus confirmed as shown in Table 1.

The structures of cephabacin $F_{4\sim6}$ and $H_{4\sim6}$ are easily deduced by assuming that some of the L-alanine fragments are substituted onto L-serine fragments from their physico-chemical properties and spectral analysis.[1] Hydrolysis of cephabacin F_4 (**16**) in basic solution afforded **8** and 3-hydroxy-4-L-serylamino-7-guanidinoheptanoic acid (**17**), $[\alpha]_D^{25}$ −16.9° (*c* 0.54, H_2O), SI-MS 306 (M+H). By the DNP method, cephabacin F_6 was found to have the L-alanine moiety as an *N*-terminal. These data confirmed the structures of cephabacin $F_{4\sim6}$ and $H_{4\sim6}$ (Table 1).

Amino acid analyses of acidic hydrolysates of cephabacin $F_{7\sim9}$ indicated the same data about known amino acids as those of the corresponding compounds, cephabacin $F_{4\sim6}$. By comparing the data of SI-MS and ^{13}C NMR spectra in these components, we assumed that cephabacin $F_{7\sim9}$ has another new amino acid which contains amino and methylene functions instead of the guanidyl group. This assignment also agreed with the negative Sakaguchi reaction in cephabacin $F_{7\sim9}$. Hydrolysis of cephabacin F_7 (**19**) under the basic condition gave **8** and 3-hydroxy-4-L-serylamino-8-aminooctanoic acid (**20**), $[\alpha]_D^{25}$ −17.0° (*c* 0.50, H_2O), SI-MS *m/z* 278 (M+H). The structure of **20** was confirmed by

proton-spin-decoupling studies (Table 2). The structures of cephabacin $F_{7\sim9}$ are given in Table 1.

The absolute configurations at the 20-C and 21-C positions were finally determined as *R* and *S*, respectively, by X-ray crystallographic analysis of *p*-bromobenzoylamino derivative of **9**, **21**, mp 214°C (dec), $[\alpha]_D^{25}$ +2.1° (*c* 1.1, MeOH) as shown in Fig. 3.

The interesting feature of these antibiotics from the viewpoint of biosynthesis is how 7-formylamination, substitution from L-alanine to L-serine or from the guanidyl group to the aminomethyl group are occurs in the cell. Also, further studies should lead to the isolation of this new family of antibiotics from other bacteria.

Experimental

The *pKa'* values, specific rotations, UV and CD spectra were measured at approx 25°C in H_2O unless otherwise stated. The IR spectra were measured in KBr pellet. The δ values in the ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra using Jeol GX-400 were recorded in ppm downfield from DSS (Sodium 2,2-dimethyl-2-silapentane-5-sulfonate). All spectra were measured in D_2O unless otherwise stated. The SI-mass spectra were measured on Hitachi M-80A mass spectrometer with xenon ion beam source. The samples were supplied by glycerol matrix.

Acetylation of **1** (**2**)

To a suspension of **1** (140 mg) in dimethylacetamide (5 ml) was added Ac_2O (1 ml) and the mixture was stirred for 2.5 hours at room temp. After removal of the solvent, the residue was loaded on preparative HPLC using YMC-Pack S-343 and eluted with 10% MeOH - 0.01 M P.B. (pH 3.0). The pure fractions detected by HPLC were collected and desalted with Diaion HP-10 (50~100 mesh, 20 ml) to give white powder of **2** (56 mg): UV λ_{max} 260 nm (ε 10,400); IR 1770, 1730, 1660, 1610, 1535 cm^{-1}.

Anal Calcd for $C_{30}H_{44}N_9O_{13}SNa\cdot 3H_2O$: C 42.50, H 5.94, N 14.87, O 22.91, S 3.78
Found: C 42.63, H 6.36, N 14.89, S 3.99

Acid Hydrolysis of **1** (**5**, **6**, **7**)

A solution of **1** (1.0 g) in 5.5 N HCl (100 ml) was refluxed for 11 hours. After evaporation of the solution, the residue was dissolved in H_2O and neutralized to pH 7.0. The solution was chromatographed on Amberlite CG-50 (H^+ type, 400 ml) and eluted with 0.1 N HCl. The fractions showing a positive Sakaguchi reaction were collected and concd. The concentrate was loaded on Diaion HP-20 (100~200 mesh, 50 ml) and eluted with H_2O to divide two fractions. The fractions eluting late were purified with preparative HPLC using YMC-Pack S-343 by the solvent system of 0.01 M P.B. (pH 6.3). The fractions giving single peak by HPLC were collected and desalted with activated carbon (10 ml) to give white powder of **5** (10 mg): IR 1730, 1685, 1665, 1550 cm^{-1}.

Anal Calcd for $C_8H_{15}N_3O_3\cdot HCl$: C 40.42, H 6.79, N 17.68, O 20.19, Cl 14.92
Found: C 40.14, H 6.56, N 17.95, Cl 13.82

The fractions eluting fast were evaporated to give crude oil, which was dissolved in 3% $NaHCO_3$ (10 ml). To the resulting solution was added benzoyl chloride (350 μl) and the mixture was stirred for 2 hours at room temp. After adjustment to pH 2, the reaction mixture was washed with OAcEt. The aqueous layer was concd and chromatographed on HP-20 (50~100 mesh, 20 ml) and eluted with 15% MeOH. Two fractions detected by HPLC were individually concd to give crude powders, **6** (130 mg) and **7** (100 mg). The crude powder (**6**) was loaded on preparative HPLC using TSK-GEL LS-410 and eluted with 8% MeOH - 0.01 M P.B. (pH 3.0). The pure fractions were desalted with Diaion HP-20 (50~100 mesh, 10 ml) to give white powder of **6** (46 mg): UV λ_{max} 226 nm (ε 11,600); IR 1650, 1580 cm^{-1}.

Anal Calcd for $C_{15}H_{22}N_4O_4\cdot 1/2H_2O$: C 54.37, H 7.00, N 16.91, O 21.72
Found: C 54.67, H 6.88, N 16.89

The crude powder (**7**) was purified with preparative HPLC using TSK-GEL LS-410 by the solvent system of 10% MeOH - 0.01 M P.B. (pH 3.0). The fractions giving single peak by HPLC were col-

lected and desalted with Diaion HP-20 (50~100 mesh, 5 ml) to give white powder of **7** (51 mg): UV λ_{max} 222 nm (ε 14,600); IR 1650, 1540 cm^{-1}.

Anal Calcd for $C_{15}H_{20}N_4O_3 \cdot 1/2H_2O$: C 57.50, H 6.75, N 17.88, O 17.87
Found: C 57.65, H 6.67, N 18.15

Alkaline Hydrolysis of **1** (**8**, **9**)

A solution of **1** (1.0 g) in 0.02 M Na_2HPO_4 (200 ml) was adjusted to pH 9.4 with 2 N NaOH. The resulting solution was stirred for 33 hours at room temp with keeping at pH 9.2~9.7. The reaction mixture was diluted with H_2O (100 ml) and adjusted to pH 7.0. The solution was applied to QAE-Sephadex A-25 (Cl^- type, 100 ml) and eluted with 0.02 M P.B. (pH 7.0). The effluent and the elution were individually desalted by activated carbon to afford crude **9** (412 mg) and pure **8** (253 mg), respectively: UV λ_{max} 258 nm (ε 9,500); IR 1770, 1675, 1615, 1520 cm^{-1}.

Anal Calcd for $C_{15}H_{19}N_4O_8SNa \cdot H_2O$: C 39.48, H 4.64, N 12.28, O 31.54, S 7.02, Na 5.04
Found: C 39.65, H 4.64, N 12.20, S 7.28, Na 5.20

The crude powder (**9**) was purified on CM-Sephadex C-25 (Na^+ type, 100 ml) with eluting 0.03 M NaCl. The pure fractions were desalted with activated carbon to afford white powder of **9** (235 mg): IR; 1665, 1550 cm^{-1}.

Anal Calcd for $C_{11}H_{23}N_5O_4 \cdot HCl \cdot H_2O$: C 38.43, H 7.62, N 20.37, O 23.27, Cl 10.31
Found: C 38.49, H 7.72, N 20.25 Cl 12.06

Similar procedures yielded hydrolysates showing below;

Starting material	Hydrolysate
10 (90 mg)	**11** (18 mg), **9** (28 mg)
16 (500 mg)	**8** (112 mg), **17** (88 mg)
19 (500 mg)	**8** (61 mg), **20** (52 mg)
A complex of cephabacin F_1 (9.0 g), F_2 (8.0 g) and F_3 (1.5 g)	**8** (3.04 g), **9** (2.2 g), **14** (1.77 g), **15** (0.71 g)

11: UV λ_{max} 258 nm (ε 8,600), IR 1760, 1630, 1605, 1530 cm^{-1}.

Anal Calcd for $C_{14}H_{18}N_3O_7SNa \cdot H_2O$: C 40.68, H 4.88, N 10.17, O 36.51, S 7.76
Found: C 40.74, H 4.87, N 10.18, S 7.96

14: IR 1670, 1550 cm^{-1}.

Anal Calcd for $C_{14}H_{28}N_6O_5 \cdot HCl \cdot H_2O$: C 40.53, H 7.53, N 20.26, O 23.13, Cl 8.55
Found: C 40.54, H 7.64, N 20.19, Cl 10.93

15: IR 1670, 1550 cm^{-1}.

Anal Calcd for $C_{17}H_3N_7O_6 \cdot HCl \cdot 1.5H_2O$: C 41.25, H 7.53, N 19.81, O 24.24, Cl 7.17
Found: C 41.76, H 8.29, N 19.26, Cl 9.34

17: IR 1675, 1560 cm^{-1}.

Anal Calcd for $C_{11}H_{23}N_5O_5 \cdot HCl \cdot H_2O$: C 36.72, H 7.28, N 19.46, O 26.69, Cl 9.85
Found: C 36.45, H 7.28, N 19.00, Cl 11.92

20: IR 1680, 1565 cm^{-1}.

Acid Hydrolysis of **9** (**12**)

A solution of **9** (500 mg) in 2 N HCl (15 ml) was refluxed for 4 hours. The concentrate residue was dissolved in H_2O at pH 4.8. The solution was chromatographed on Dowex 50WX2 (Na^+ type, 50~100 mesh, 30 ml) and eluted with 2 M NaCl. The fractions showing a positive Sakaguchi reaction were loaded on activated carbon and eluted with H_2O and followed by 40% MeOH. The pure fractions detected by HPLC were collected and freeze-dried to give white powder of **12** (206 mg): IR 1670, 1570 cm^{-1}.

Anal Calcd for $C_8H_{15}N_4O_3 \cdot HCl \cdot 1/2H_2O$: C 36.43, H 7.64, N 21.25, O 21.24, Cl 13.44
Found: C 36.01, H 7.66, N 21.11, Cl 14.24

Acetylation of **12** (**13**)

To a solution of **12** (200 mg) in 3% $NaHCO_3$ (10 ml) was added Ac_2O (370 μl) and the mixture was stirred for 1 hour at room temp. The reaction mixture was desalted with activated carbon

and purified with preparative HPLC using YMC-Pack SH-343 by the solvent system of 0.01 M **P.B.** (pH 3.0). The fractions giving single peak by HPLC were collected and desalted with activated carbon to afford **13** (96 mg) as white powder: IR 1650, 1560 cm^{-1}.

Anal Calcd for $C_{10}H_{20}N_4O_4 \cdot 1/2H_2O$: C 44.60, H 7.86, N 20.81, O 26.73
Found: C 44.20, H 7.81, N 20.79

p-Bromobenzoylation of **9** (**21**)

To a solution of **9** (1.0 g) in 3% $NaHCO_3$ (140 ml) was added *p*-bromobenzoyl chloride (1.0 g) and the mixture was stirred for 6 hours at room temp. After adjustment to pH 2, the reaction mixture was washed with EtOAc. The aqueous layer was neutralized to pH 7 and concd. The concentrate was chromatographed on HP-20 (50~100 mesh, 20 ml) and eluted with 50% MeOH. The pure fractions detected by HPLC were concd to give **21** (1.09 g) as white powder. The powder (500 mg) was dissolved in a small amount of H_2O and stood at room temp to yield crystals (365 mg) of **21**: UV λ_{max} 243 nm (ε 17,900); IR 1675, 1650, 1595, 1550 cm^{-1}.

Anal Calcd for $C_{18}H_{26}N_5O_5Br \cdot 1/2H_2O$: C 44.92, H 5.65, N 14.55, O 18.28, Br 16.60
Found: C 44.77, H 6.14, N 14.26, Br 16.90

X-Ray Crystallographic Analysis of **21**

The crystal belongs to the monoclinic system with the space group C2 and the unit-cell dimensions are a=22.368(5), b=13.742(4), c=14.347(4) Å, β=93.22(2)° and V=4403(2) $Å^3$. The calculated density (Dx=1.45 $g \cdot cm^{-3}$) suggests z=8, thus requiring that the asymmetric unit consists of two independent molecules. Reflection data were obtained with a Rigaku AFC-5 diffractometer using MoKα radiation (λ=0.7107 Å). Out of 3395 independent reflections, 2466 reflections had $F \geqq 3\delta$ (F), and therefore were used in the calculations for structure determination. Atomic coordinates of 60 non-hydrogen atoms including two water molecules were determined using direct method.[9] Atomic coordinates and anisotropic temperature factors were refined by the least-square method[10] to an R-value of 0.085.

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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