PB-5266 A, B AND C, NEW MONOBACTAMS

II. PHYSICO-CHEMICAL PROPERTIES AND CHEMICAL STRUCTURES

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(Received for publication August 12, 1986)

The chemical structures of three new monobactams, PB-5266 A, B and C, were elucidated by their physico-chemical properties and spectrometric studies. In contrast to previously described monobactams, they all possess a dehydroasparagine residue.

PB-5266 A (**1**), B (**2**) and C (**3**) were isolated and purified from the culture broth of *Cytophaga johnsonae* PB-5266 as described in a previous paper.[1]

Their physico-chemical properties are summarized in Table 1. The IR spectra of PB-5266 A, B and C (Fig. 1) exhibited characteristic bands at 1760 (β-lactam carbonyl), 1720 (α,β-unsaturated carbonyl), 1265~1240 and 1050 cm^{-1} (sulfamic acid). The characteristic bands at 1760, 1265~1240 and 1050 cm^{-1} and strongly acidic character indicated these antibiotics to be monobactams. The UV spectra of these antibiotics exhibit a maximum at 261~263 nm, which has not been observed in the known monobactams. On acid hydrolysis PB-5266 A, B and C produced 2,3-diaminopropionic acid (Dpr). This indicated these antibiotics were non-methoxylated monobactams.[2,3]

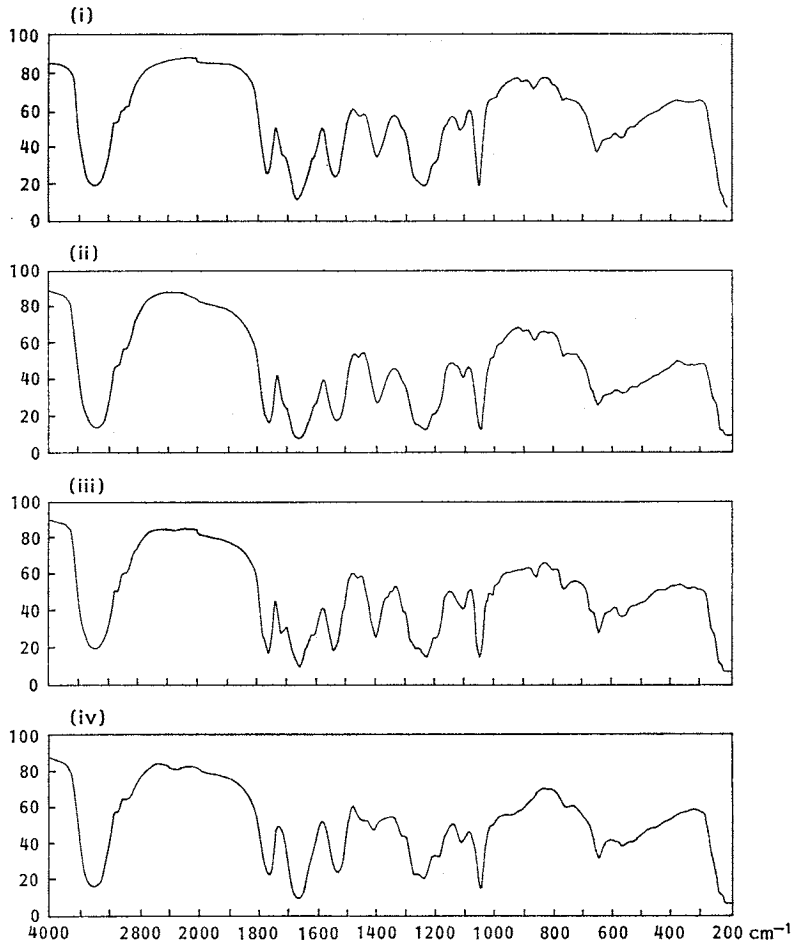
On acid hydrolysis **1** produced Ala, Dpr and glyceric acid. From the ^1H NMR and ^{13}C NMR studies the presence of olefinic group was suggested. When **1** was hydrogenated on platinum, a hydrogenated product (**4**) was obtained. On acid hydrolysis, **4** gave Asp in addition to Ala, Dpr and glyceric acid. From this result the presence of dehydroaspartic residue in **1** was indicated. In the IR spectrum of **4** (Fig. 1), the absorption at 1720 cm^{-1} in **1** disappeared. In the UV spectrum **4** showed only end absorption. These results also suggested the presence of dehydroaspartic acid

Table 1. Physico-chemical properties of PB-5266 A (**1**), B (**2**), C (**3**) and the hydrogenated product of A (**4**).

	PB-5266 A (**1**)	PB-5266 B (**2**)	PB-5266 C (**3**)	Hydrogenated product of A (**4**)
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless powder
Nature	Acidic	Acidic	Acidic	Acidic
Molecular formula	$C_{13}H_{18}N_5O_{10}SK$	$C_{13}H_{18}N_5O_{11}SK$	$C_{12}H_{16}N_5O_{10}SK$	$C_{13}H_{20}N_5O_{10}SK$
SI-MS (m/z)	514	530	500	516
UV $\lambda_{max}^{20mM\ KH_2PO_4}$ ($E_{1cm}^{1\%}$)	262 (262)	261 (228)	261 (234)	End absorption
IR ν_{max}^{KBr} cm^{-1}	1760, 1720, 1310, 1260~1210	1760, 1720, 1310, 1260~1210	1760, 1720, 1310, 1260~1210	1760, 1310 1260~1210
Amino acid analysis (found μmol/mg)	Ala (1.81) Dpr† (+)	Ser (1.76) Dpr† (−)	Gly (2.21) Dpr† (+)	Ala (1.51) Dpr† (+) Asp (1.51)

† Dpr could not be detected because of the overlap with the peak of ammonia on amino acid analysis. Thus it was detected on HPLC after dansylation.

Fig. 1. IR spectra of PB-5266 A (i), B (ii), C (iii) and the hydrogenated product of A (iv).



residue in **1**.

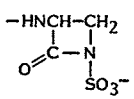
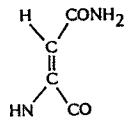
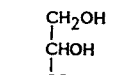
The molecular formula, $C_{13}H_{18}N_5O_{10}SK$, was suggested by secondary ion (SI)-MS and elemental analysis of potassium salt of **1** (*Anal* Calcd for $C_{13}H_{18}N_5O_{10}SK \cdot 2H_2O$: C 30.52, H 4.34, N 13.60, S 6.27. Found: C 30.68, H 4.28, N 13.38, S 5.82). This indicated dehydroaspartic acid residue was present in amide form (namely as dehydroasparagine residue).

All signals in the ^1H NMR and ^{13}C NMR of **1** could be assigned exactly as shown in Table 2.

The sequence of the four constituents of **1** was determined by ^{13}C-^1H long-range coupling constants as shown in Fig. 2. The coupling between methine proton of Dpr and carbonyl carbon of dehydroasparagine residue and that between methine proton of Ala and carbonyl carbon of glyceric acid were observed. The position of glyceric acid residue at *N*-terminus was suggested by negative reaction to ninhydrin test.

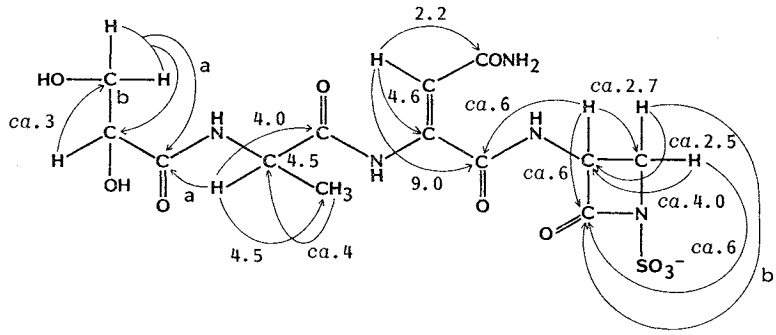
From the acid hydrolysate of **1**, glyceric acid was isolated and subjected to CD measurement. From the comparison with the authentic sample, the absolute configuration was determined to be *R*. The amino acid mixture obtained from the acid hydrolysis of **1** was L-leucylated by the conventional procedure and followed by analysis on HPLC.[4] The result indicated the absolute configura-

Table 2. ^{1}H and ^{13}C NMR of PB-5266 A, B and C.

		PB-5266 A (1)				PB-5266 B (2)				PB-5266 C (3)		
		δ_H	J (Hz)	δ_C		δ_H	J (Hz)	δ_C		δ_H	J (Hz)	δ_C
	β-CH_2	3.78 (dd)	3.0, 5.9	47.6		3.79 (dd)	3.0, 5.9	47.7		3.79 (dd)	3.0, 5.9	47.8
		3.93 (t)	5.9			3.94 (t)	5.9			3.94 (t)	5.9	
	α-CH	5.00 (dd)	3.0, 5.9	56.0		5.01 (dd)	3.0, 5.9	56.1		5.00 (dd)	3.0, 5.9	56.0
	CO			166.2				166.2				166.2
	β-CO			169.6				169.6				169.7
	β-HC=	6.37 (s)		110.1		6.43 (s)		110.5		6.41 (s)		109.8
	α-C=			141.0				140.7				140.9
	α-CO			167.4				167.3				167.4
	Ala				Ser				Gly			
Amino acid	β-CH_3	1.42 (d)	7.2	17.2	β-CH_2	4.53 (t)	5.0	61.8	α-CH_2	4.07 (s)		43.3
residue	α-CH	4.39 (q)	7.2	50.6	α-CH	3.91 (d)	5.0	56.4				
	CO			174.3	CO			171.2	CO			170.6
	β-CH_2	3.78 (d)	4.3	64.1		3.81 (d)	4.3	64.1		3.82 (d)	4.3	64.2
	α-CH	4.25 (t)	4.3	72.9		4.31 (t)	4.3	73.1		4.30 (t)	4.3	73.1
	CO			175.2				175.5				176.1

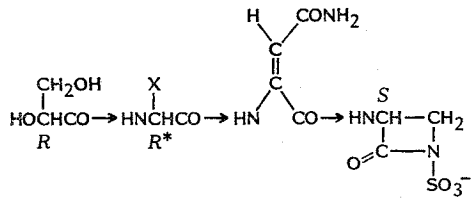
^{1}H NMR are taken in D_2O using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt hydrate (DSS) as an internal reference.
^{13}C NMR are taken in D_2O using acetonitrile as an internal reference.

Fig. 2. ^{13}C-^{1}H Long-range coupling constants found in PB-5266 A (1) (Hz in D_2O at 24°C).



a: Coupling constants could not be measured because of multiplicity.
b: Coupling constant <2 Hz.

Fig. 3. Total structures of PB-5266 A (1), B (2) and C (3).



PB-5266 A X = CH_3
PB-5266 B X = CH_2OH
PB-5266 C X = H

* Except PB-5266 C.

tion of Ala and Dpr to be *R* and *S* respectively. The ^{13}C-^{1}H long-range coupling constant ($^{1}J_{C-H}$= 9.0 Hz) between β-methine proton and α-carbonyl carbon of dehydroasparagine residue suggests the geometry of olefinic bond to be *E*. Thus the total structure of PB-5266 A was determined as shown in Fig. 3.

As supposed from the result described above, the hydrogenated product (4) was a mixture of two diastereoisomers as shown in Fig. 4. The NMR data listed in Table 3 confirmed it.

PB-5266 B (2) and C (3) showed almost the same physico-chemical properties, although Ser was found in 2 and Gly in 3 instead of Ala in 1 (Table 1). NMR and SI-MS data suggest Ser in 2 and Gly in 3 were present in the position of Ala in 1. The absolute configuration of the constituent amino acids in 2 and 3 was determined in the same procedure as 1. Thus the structures of 2 and 3 were elucidated as shown in Fig. 3.

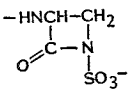
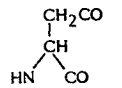
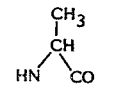
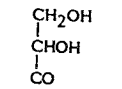
Experimental

^{1}H and ^{13}C NMR spectra were recorded with a Varian XL-200 spectrometer. IR absorption spectra were measured with a Jasco DS-403G spectrometer, and CD spectra with a Jasco J-40C automatic recording spectropolarimeter. SI-MS spectra were taken with a Hitachi M-68 mass spectrometer. Amino acid analysis was carried out with a Hitachi amino acid autoanalyzer 835 under the normal condition directed for the instruments.

Hydrogenation of PB-5266 A

Platinum oxide was suspended in water and stirred under hydrogen atmosphere for 30 minutes.

Table 3. ^{1}H and ^{13}C NMR of the hydrogenated product (4).

		δ_H	J (Hz)	δ_C	
	β-CH_2	a 3.71 (dd)	3.1, 5.8	48.3*	
		3.91 (t)	5.8		
		b 3.67 (dd)	3.1, 5.8		
		3.91 (t)	5.8		
	α-CH	a 4.96 (dd)	3.1, 5.8	55.9*	
		b 4.94 (dd)	3.1, 5.8		
	CO			166.8*	
	β-CH_2	a 2.72 (dd)	8.2, 15.7	37.0	
		2.85 (dd)	5.6, 15.7		
		b 2.74 (dd)	8.0, 15.5	36.8	
		2.84 (dd)	5.8, 15.5		
	α-CH	a 4.75 (dd)	5.6, 8.2	51.1*	
		b 4.70 (dd)	5.8, 8.0		
	β-CH_3	a 1.39 (d)	7.0	17.3	
		b 1.40 (d)	7.2	17.2	
	α-CH	4.38 (q)*	*ca.* 7	a 50.5	b 50.4
	β-CH_2	3.79 (d)*	4.3	64.1*	
	α-CH	4.26 (t)*	4.3	a 72.9	b 73.0
CO				a 173.4	b 173.2
				a 175.2	b 175.3
				a 175.3	b 175.5
				a 175.8	b 175.6

* Signals of two diastereoisomers overlap.

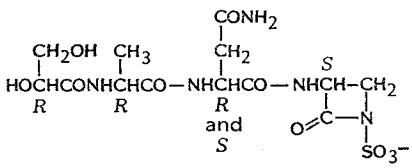
Two diastereoisomers are depicted as a and b.

^{1}H NMR are taken in D_2O at 45°C using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt hydrate (DSS) as an internal reference.

^{13}C NMR are taken in D_2O using acetonitrile as an internal reference.

The aqueous solution of PB-5266 A (40 mg) was added and stirred under hydrogen atmosphere for 7 hours. Platinum was filtered off, and the filtrate was freeze-dried. The obtained colorless powder (38 mg) was charged on a column of Biogel P-2 and eluted with 1% butanol. Freeze-dry of the active eluates gave colorless powder of the hydrogenated product (31 mg).

Fig. 4. Structure of the hydrogenated product (4).



Isolation of Glyceric Acid from PB-5266 A

PB-5266 A potassium salt (11 mg) was hydrolyzed with constantly boiling hydrochloric acid at 110°C for 3 hours. The hydrolysate was concentrated to dryness, and the residue was extracted with ethyl acetate repeatedly. The extract was purified by preparative TLC on Silica gel plates (Merck) with $CHCl_3$ - EtOH - 14% NH_4OH (4 : 7 : 2). The zone of glyceric acid (Rf 0.15) was detected by spraying $KMnO_4$ solution and extracted with 50% aqueous methanol. The extract was acidified and dried, and then extracted with ethyl acetate to obtain free form of glyceric acid (1.7 mg).

CD: $[\theta]_{255}$ 0, $[\theta]_{238}$ +31, $[\theta]_{232.5}$ 0, $[\theta]_{206}$ −1,060, $[\theta]_{200}$ −870 (*c* 0.170, 50 mM phosphate buffer, pH 7.0)

A commercially available D-glyceric acid calcium salt monohydrate gave the following CD spectrum.

CD: $[\theta]_{240}$ 0, $[\theta]_{207}$ −670, $[\theta]_{200}$ −310 (*c* 0.0256, 50 mM phosphate buffer, pH 7.0).

References

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