Communications to the editor

ASPARENOMYCIN A, A NEW CARBAPENEM ANTIBIOTIC

Sir:

In the course of our screening work for new β-lactam antibiotics, we found that a streptomycete numbered PA-31088, which was identified as a new species and named *Streptomyces tokunonensis* sp. nov., produces a new carbapenem antibiotic named asparenomycin A* and some related antibiotics. These antibiotics were also found in the culture broth of another strain PA-39504 identified as *Streptomyces argenteolus*.

The strain PA-31088 was fermented in 30-liter jar fermentor containing 20 liters of a medium composed of 2.4% tomato paste, 2.4% dextrin, 1.2% dry yeast, 0.0006% $CoCl_2 \cdot 6H_2O$, at 28°C under agitation of 300 rpm and aeration of 20 liters per minute for 65 hours.

The antibiotics in the culture filtrate (100 liters) were adsorbed on a column of Amberlite IRA-68 (Cl^-) (Rohm and Haas Co., Ltd.) and eluted with 5% NaCl. The active eluate was desalted on a Diaion HP-20 (Mitsubishi Kasei Kogyo Co., Ltd.) column and then adsorbed on an activated carbon at pH 5.0 and eluted with 60% acetone (pH 7.0). Evaporation and lyophilization of the eluate gave a crude powder (20 g). Isolation of asparenomycin A was achieved by successive column chromatography on a Pre PAK-500/C_{18} column of a High Speed Liquid Chromatography System 500 (Waters Co., Ltd.) and a column of a Diaion HP-20AG (200~400 mesh) with phosphate buffer solution, pH 7.0. The fraction of asparenomycin A from the last column was desalted on an HP-20 column and lyophilized. A substantially pure preparation of the antibiotic was obtained as the sodium salt (210 mg).

Asparenomycin A sodium salt is a colorless amorphous powder, which gradually decomposes above 150°C, and is soluble in water, methanol and dimethylsulfoxide but insoluble in ethyl acetate, acetone and chloroform. It shows positive color reaction to EHRLICH's reagent but is ninhydrin-negative. On paper electrophoresis in 50 mM phosphate buffer, pH 7.0 at 10 volt/cm for 3 hours, the antibiotic moved towards the anode with the same mobility as that of penicillin N. Elemental analysis (Found: C, 45.30; H, 4.82; N, 7.74; S, 7.86; Na, 5.99) and FD mass: 355 (MH^+ of the methyl ester) agreed with a molecular formula $C_{14}H_{16}N_2O_6S$ for the free acid. The antibiotic shows UV (10 mM phosphate buffer, pH 7.0), λ_{max}: 241 nm (ε, 21472), 280 nm

Table 1. ^1H NMR spectra of **I** and **II** sodium salts.

Assignment	δ ppm (J Hz)	
	I	**II**
9-CH_3	1.99 (s)	1.85 (s)
17-CH_3	2.12 (s)	2.16 (s)
		2.17 (s)
4-CH_2	3.16 (d-like)	2.0~2.9 (m)
5-CH	5.01 (t-like)	4.6~5.4 (m)
3-CH	—	
10-CH_2	4.26 (s)	4.23 (s)
		4.24 (s)
13-CH	6.34 (d, 14.0)	6.11 (d, 14.0)
		6.22 (d, 14.1)
14-CH	7.53 (d, 14.0)	7.50 (d, 14.0)
		7.61 (d, 14.1)

Spectra were recorded with a Varian XL-100-12A spectrometer in D_2O at room temperature using DSS as an internal reference.

Table 2. ^{13}C NMR spectrum of **I** sodium salt.

Assignment (Type)	δ ppm	Assignment (Type)	δ ppm
9 (-CH_3)	15.9 (q)	14 (=CH-)	134.7 (d)
17 (-CH_3)	23.0 (q)	3[a] (=C<)	135.6 (s)
4 (-CH_2-)	32.5 (t)	2[a] (=C<)	143.2 (s)
5 (>CH-)	60.4 (d)	8 (=C<)	150.3 (s)
10 (-CH_2-)	64.5 (t)	7 (O=C<)	166.7 (s)
13 (=CH-)	111.7 (d)	16 (O=C<)	173.2 (s)
6[a] (=C<)	134.2 (s)	11 (O=C<)	174.6 (s)

Spectrum was recorded with a Varian XL-100-12A spectrometer in D_2O at 5°C using CH_3CN as an internal reference. δ: Calculated by assuming δ (CH_3CN)=1.7 ppm from DSS.

[a] Tentative assignments.

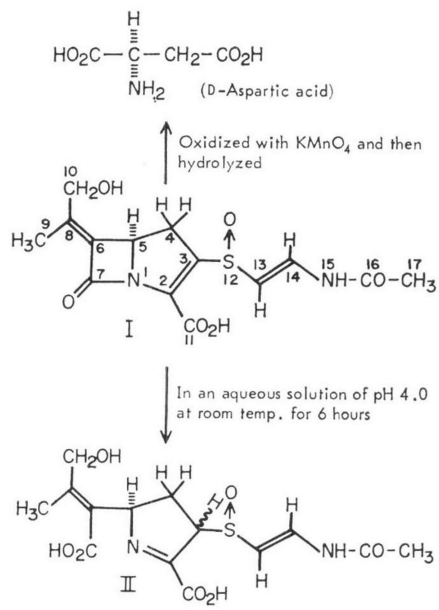
* Presented in Japan Kokai (patent) 55-13,628 (Aug. 10, 1980) as a name PA-31088-IV.

(shoulder), 320 nm (shoulder); IR (KBr), 1750, 1695, 1620, 1380, 1270, 1200, 1045, 950 cm^{-1}; $[\alpha]_D^{22}$ $-210.8\pm5.1°$ (*c* 0.536, 10 mM phosphate buffer, pH 7.0); CD: $[\theta]_{390}$ 0, $[\theta]_{315}$ −22754, $[\theta]_{278}$ −102702, $[\theta]_{258.5}$ 0, $[\theta]_{243}$ +93292, $[\theta]_{190}$ +14480 (*c* 0.0458, 10 mM phosphate buffer, pH 7.0). The signals and assignments of ^1H NMR and ^{13}C NMR spectra are shown in Tables 1 and 2.

Interpretation of these physico-chemical properties and chemical behaviors described below lead to the structure of asparenomycin A as **I** in Scheme 1.

In an aqueous solution of pH 4.0, asparenomycin A was hydrolyzed to a non β-lactam compound (**II**) (the absorption at 1750 cm^{-1} disappeared) at room temperature within 6 hours. Most peaks of the ^1H NMR spectrum of **II** were observed as pairs, suggesting that **II** is a mixture of diastereoisomers caused by appearance of a new additional proton (Table 1).[1] **II** moves two times faster than penicillin N in the paper electrophoresis. **II** shows a positive EHRLICH reaction but a negative ninhydrin reaction. When an aqueous solution of **II** of pH 2.0 was allowed to stand at room temperature for 16 hours, no significant changes were detected by thin-layer chromatography and by paper electrophoresis. A high possibility of E orientation at C–8 was indicated by the fact that **II** does not give detectable γ-lactone. When asparenomycin A was

Scheme 1.



oxidized with $KMnO_4$ and then hydrolyzed with 6 N HCl at 110°C for 2 hours, a significant amount of aspartic acid was detected by an automatic amino acid analyzer. The configuration of the amino acid was determined to have D-configuration from the result of HPLC of its L-leucylated product. Consequently the *R* configuration at 5-C is concluded.

Table 3. Antibacterial activity of asparenomycin A, ampicillin and cefoxitin.

Organism	MIC (μg/ml)[a]		
	Asparenomycin A	Ampicillin	Cefoxitin
Staphylococcus aureus 209P JC-1	1.56	0.1	1.56
Staphylococcus aureus C-14[b]	1.56	6.25	3.13
Streptococcus pyogenes C-203	1.56	0.05	0.78
Escherichia coli NIHJ JC-2	1.56	6.25	3.13
Escherichia coli 377[c]	0.39	100	12.5
Klebsiella pneumoniae SRL-1	0.78	0.78	1.56
Klebsiella sp. 363[b]	0.78	>100	1.56
Proteus mirabilis PR-4	3.13	1.56	1.56
Proteus vulgaris CN-329	12.5	50	3.13
Enterobacter cloacae 233	1.56	50	>100
Serratia marcescens ATCC 13880	12.5	25	12.5
Pseudomonas aeruginosa ATCC 25619	25	>100	>100

[a] Determined by agar dilution method in MUELLER-HINTON agar and inoculated by one loopful of *ca.* 10^6 cells per ml.
[b] Penicillinase producing strain.
[c] Cephalosporinase producing strain.

Table 4. Inhibition of β-lactamases produced by Gram-negative bacteria by asparenomycin and clavulanic acid.

Source of β-lactamase[a]	Class[b]	Minimum effective concentration (μg/ml)[c]	
		Asparenomycin A	Clavulanic acid
Escherichia coli 6	Ib	1.0	250
Enterobacter cloacae 92	Ia	1.0	>250
Proteus vulgaris 31	Ic	0.001	1
Escherichia coli W3110 RTEM	IIIa	0.016	0.063
Klebsiella sp. 363	IV	0.063	0.063
Enterobacter cloacae 53	IVa	0.25	0.063

[a] Enzyme preparations used were partially purified.
[b] Classification of RICHMOND and SYKES.
[c] Inhibitor was incubated with enzyme at 30°C for 10 minutes prior to adding nitrocefin (50 μg/ml) and minimum concentration to inhibit color change was determined.

The complete structure of asparenomycin A including the orientation of sulfoxide (*R*) was confirmed by X-ray crystallographic analysis[2] of the *p*-nitrobenzyl ester and the methyl ester of the antibiotic.

Asparenomycin A is unique in having the substituted ethylidene side chain in the structure and is obviously distinguished from hitherto known carbapenem antibiotics such as thienamycin, olivanic acid derivatives, PS-5 and carpetimycins A and B.[3]

Asparenomycin A is active against a broad range of Gram-positive and Gram-negative bacteria including β-lactamase producing organisms as shown in Table 3. It shows strong inhibitory activity against various type of β-lactamases as shown in Table 4.

Acknowledgements

The authors wish to thank to Drs. Y. NAKAGAWA and Y. IKENISHI of our laboratories for the measurement of FD mass spectra.

KENTARO TANAKA
JUN'ICHI SHOJI
YOSHIHIRO TERUI
NAOKI TSUJI
EIJI KONDO
MIKAO MAYAMA
YOSHIMI KAWAMURA
TERUO HATTORI
KOICHI MATSUMOTO
TADASHI YOSHIDA

Shionogi Research Laboratories
Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

(Received April 23, 1981)

References

1) MAEDA, K.; S. TAKAHASHI, M. SEZAKI, K. IINUMA, H. NAGANAWA, S. KONDO, M. OHNO & H. UMEZAWA: Isolation and structure of a β-lactamase inhibitor from *Streptomyces*. J. Antibiotics 30: 770~772, 1977
2) Will be published elsewhere.
3) NAKAYAMA, M.; A. IWASAKI, S. KIMURA, T. MIZOGUCHI, S. TANABE, A. MURAKAMI, I. WATANABE, M. OKUCHI, H. ITOH, Y. SAINO, F. KOBAYASHI & T. MORI: Carpetimycins A and B, new β-lactam antibiotics. J. Antibiotics 33: 1388~1390, 1980 and references cited therein.