NEW ANTIBIOTICS, CLAZAMYCINS A AND B

Sir:

Two chlorine-containing antibiotics, clazamycins A and B have been isolated from the culture broth of *Streptomyces* No. MF990-BF4 which is closely related to *Streptomyces violaceorectus*[1] and *Streptomyces cinereoruber*[2].

The strain was cultured at 27°C for 67 hours on a rotatory shaker (180 r.p.m.) in a medium containing 1.5% glycerol, 1.5% cotton seed meal, 0.3% NaCl and 0.2% L-asparagine (adjusted to pH 7.4). Vegetative inoculum, 3% by volume, grown for 24 hours in the same medium was used. The culture broth in 45 flasks was collected and filtered (4.6 liters, 76 μg/ml of clazamycins) using Hyflo Supercel as the filter aid. Concentrations of clazamycins were determined by the usual cylinder plate method against *Pseudomonas aeruginosa* No. 12, using crystalline clazamycin A hydrochloride (826 μg/mg) as the assay standard.

The antibiotics in the filtrate were adsorbed on a column of Amberlite IRC-50 (70% Na^+ form, 400 ml) and eluted with 0.5 N hydrochloric acid. The active neutral eluate (945 ml) was concentrated to dryness (14.9 g) and the residue was extracted with 60 ml of methanol. The extract was concentrated and dissolved in 70 ml of water. The antibiotics in the aqueous solution were purified by column chromatographies on Amberlite XAD-2 (180 ml) and on activated carbon (17 ml) eluting with water. The active eluate was concentrated to dryness yielding a mixture (314 mg, 558 μg/mg) of clazamycins A and B (hydrochlorides).

The mixture was dissolved in 1.5 ml of water and adsorbed on a column of Amberlite XAD-2 (300 ml). The column was eluted with water and the eluate was cut into each 10-ml fractions. Fractions 22~26 were combined and concentrated to dryness yielding a colorless powder (124 mg, 593 μg/mg) of clazamycin B hydrochloride. The hydrochloride was further purified by rechromatography on Amberlite XAD-2, yielding pure clazamycin B hydrochloride (660 μg/mg). Fractions 27~45 were combined and concentrated to yield pure clazamycin A hydrochloride (83 mg, 826 μg/mg) which crystallized in a mixture of ethanol and ethyl acetate.

Clazamycin A hydrochloride is obtained as colorless crystals melting at 110°C with decomposition. $[\alpha]_D^{25}$ −56° (*c* 1.0, water). Anal calcd. for $C_7H_9N_2OCl \cdot HCl$: C 40.21, H 4.82, N 13.39, Cl 33.91. Found: C 40.12, H 4.80, N 13.26, Cl 34.06. The molecular formula is also derived by the high-resolution MS spectrum (calcd. mol. wt. for $C_7H_9N_2OCl$, 172.0402; found *m/e* 172.0401). The hydrochloride shows UV maxima at 212 nm (ε 11,800) and 250 nm (ε 2,600) in an aqueous solution.

Clazamycin B hydrochloride obtained as a colorless hygroscopic powder shows no definite melting point. $[\alpha]_D^{24}$ +96° (*c* 1.0, water). The same molecular formula is derived by the high-resolution MS spectrum (calcd. mol. wt. for $C_7H_9N_2OCl$, 172.0402; found *m/e* 172.0422). The hydrochloride shows UV maxima at 212 nm (ε 9,500) and 250 nm (ε 1,830) in an aqueous solution.

The IR spectra of clazamycins A and B are represented in Figs. 1 and 2, respectively. The PMR and CMR chemical shifts of clazamycins A and B are shown in Tables 1 and 2. Both clazamycins A and B give positive RYDON-SMITH and pentacyanoaquoferriate reactions and negative ninhydrin reaction. Their hydrochlorides are soluble in water and lower alcohols. By high-voltage paper electrophoresis with 3,500 V for 15 minutes in formic acid - acetic acid - water (1 : 3 : 36, v/v), both antibiotics move to the cathode with Rm (relative mobility to alanine) 1.10. Clazamycins A (Rf 0.40) and B (Rf 0.27) can be separated by thin-layer chromatography using Silica gel G (Merck, Art. 5715) with butanol - acetic acid - water (6 : 1 : 2, v/v).

Clazamycin A is stable in an aqueous solution at pH 2.2 for 24 hours at room temperature, but

Table 1. PMR chemical shifts of clazamycins A and B (hydrochlorides)

Proton	δ ppm (J Hz)	
	Clazamycin A	Clazamycin B
1-H	7.95 d (6.0)	7.94 d (5.9)
2-H	7.07 d (6.0)	7.03 d (5.9)
4-H_2	4.29 q (5.0, 13)	4.42 q (3.1, 13)
	4.69 q (6.3, 13)	4.79 q (7.2, 13)
5-H	5.59 m	5.62 m
6-H_2	2.82 q (7.5, 14)	3.05 d (4.5)
	3.28 q (6.3, 14)	

Chemical shifts, δ (ppm) were measured in D_2O using TMS as the external reference.

clazamycin B is converted to a mixture of A and B (1: 3). An equilibrium conversion of clazamycins was analyzed by thin-layer chromatographic technique. Clazamycin A or B was converted to a mixture of A and B (3: 2) in an aqueous solution at >pH 6.8 for 24 hours at room temperature.

The structure of clazamycin A was determined to be (5*S*,6a*R*)-5-chloro-4,5,6,6a-tetrahydro-6a-hydroxy-3-imino-3*H*-3a-azapentalene by X-ray crystallographic analysis of its hydrochloride as described in a following paper[3]. PMR and CMR signals of clazamycin A can reasonably be assigned as shown in Tables 1 and 2, respectively. The structure of clazamycin B is confirmed to be a 6a-epimer of clazamycin A by spectral data of its hydrochloride (Tables 1 and 2) and by equilibrium conversion of clazamycins.

Clazamycins A and B have weak antibacterial activities, as shown in Table 3. In the treatment with daily intravenous doses of 12.5~100 μg of clazamycin A per mouse for 10 days, more than 130% prolongations in the survival period of mice inoculated with leukemia L-1210 cells were ob-

Table 2. Carbon-13 chemical shifts of clazamycins A and B (hydrochlorides) in D_2O.

Carbon	Chemical shifts (δ)	
	Clazamycin A	Clazamycin B
1	154.0 d	153.4 d
2	123.3 d	123.2 d
3	169.0 s	167.9 s
4	53.8 t	55.0 t
5	60.1 d	60.4 d
6	43.2 t	42.3 t
6a	104.2 s	105.0 s

δ: ppm from TMS using dioxane (δ=67.4 ppm) as the internal reference.
s,d,t: Multiplicity of off-resonance.

Fig. 1. The IR spectrum of clazamycin A hydrochloride in KBr.

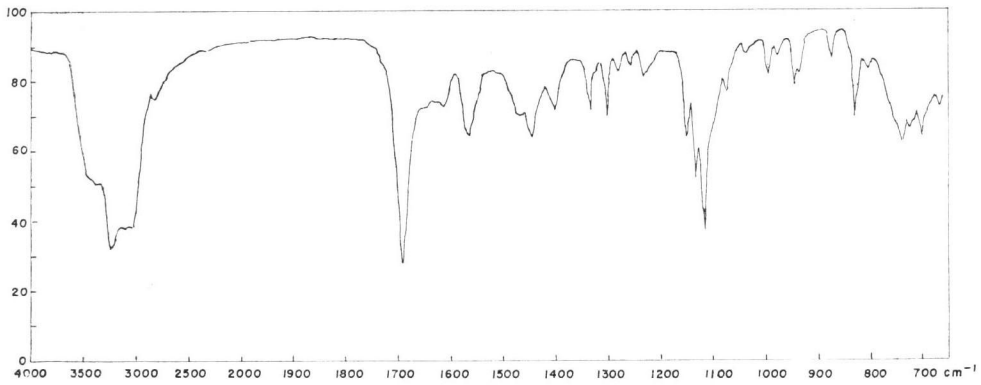


Fig. 2. The IR spectrum of clazamycin B hydrochloride in KBr.

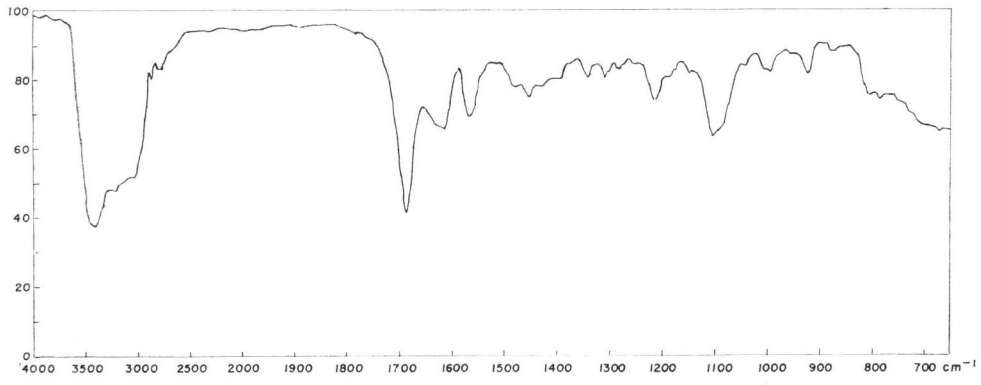
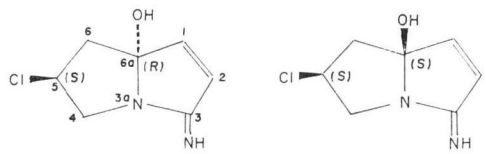


Table 3. The antimicrobial spectra of clazamycins (hydrochloride).

Test organisms	Minimum inhibitory concentrations (μg/ml)		Test organisms	Minimum inhibitory concentrations (μg/ml)	
	Clazamycin A	Clazamycin B		Clazamycin A	Clazamycin B
Staphylococcus aureus FDA209P	100	100	*Klebsiella pneumoniae* PCI602	25	50
Staphylococcus aureus Smith	100	100	*Klebsiella pneumoniae* 22#3038	50	100
Micrococcus flavus FDA16	25	25	*Shigella dysenteriae* JS11910	25	25
Sarcina lutea PCI1001	100	100	*Shigella flexneri* 4b JS11811	12.5	25
Bacillus anthracis	6.25	12.5	*Shigella sonnei* JS11746	25	25
Bacillus subtilis PCI219	100	100	*Salmonella typhi* T-63	25	12.5
Bacillus subtilis NRRL B-558	100	100	*Salmonella enteritidis* 1891	25	50
Corynebacterium bovis 1810	100	100	*Proteus vulgaris* OX19	25	25
Escherichia coli NIHJ	100	100	*Pseudomonas aeruginosa* A3	25	12.5
Escherichia coli K-12	50	50	*Pseudomonas aeruginosa* No. 12	100	100
Escherichia coli ML 1629	50	50	*Pseudomonas aeruginosa* TI-13	50	50
Escherichia coli ML 1410	50	50	*Pseudomonas aeruginosa* GN315	50	25
Escherichia coli ML 1410 R81	50	50	*Pseudomonas aeruginosa* K-Ps102	25	50
Escherichia coli W677	50	50	*Pseudomonas maltophilia* GN907	50	50
Escherichia coli JR66/W677	50	50			

Bacteria were incubated on a 0.5% peptone agar plate at 37°C for 17 hours.



Clazamycin A Clazamycin B

served. Intravenous acute LD_{50} of clazamycins A and B in mice were 50~100 mg/kg and >100 mg/kg, respectively.

Dihydroclazamycin A (*m/e* 174) prepared by hydrogenation of clazamycin A with palladium on carbon in water has no antibacterial activity.

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