Communications to the editor

OGANOMYCIN A, A NEW CEPHAMYCIN-TYPE ANTIBIOTIC PRODUCED BY *STREPTOMYCES OGANONENSIS* AND ITS DERIVATIVES, OGANOMYCINS B, GA AND GB

Sir:

In a previous paper[1] we have reported that *Streptomyces oganonensis* No. Y-G19Z produced oganomycin G when 5-mercapto-1-methyl-1*H*-tetrazole was added to the fermenting broth. In the course of fermentation studies on oganomycin G, we found a new cephamycin-type antibiotic[2]. The antibiotic was named oganomycin A. Its structure was determined to be 7β-(D-5-amino-5-carboxyvaleramido)-7α-methoxy-3-(*p*-sulfooxycinnamoyloxymethyl)-Δ^3-cephem-4-carboxylic acid by spectrometric and degradation studies. Production of oganomycin A was greatly stimulated by the addition of *p*-hydroxycinnamic acid to the medium.

Oganomycin A was produced when *Streptomyces oganonensis* No. Y-G19Z was grown at 30°C in a medium containing 7 % starch, 2 % gluten meal, 2 % soybean meal, 0.8 % glycerol, 0.1 % casamino acids and 0.3% ferric sulfate with or without the addition of 0.3 % sodium *p*-hydroxycinnamate (pH 7.5). After five days of fermentation when the oganomycin A level, assayed by a high performance liquid chromatography (HPLC), reached a maximum, the broth was harvested and filtered. Oganomycin A in the filtrate was adsorbed on Diaion HP-20 at pH 3.0 and eluted with 30 % aqueous acetone. The antibiotic-containing active fractions were collected and concentrated under reduced pressure. Oganomycin A in them was adsorbed on Amberlite IRA-68 (Cl^-) and eluted with 1 M sodium nitrate at pH 7.2 containing 0.1 M sodium acetate. After repetition of HP-20 adsorption and acetone elution as above stated, the active fractions were chromatographed on a cellulose powder (Avicel) equilibrated and eluted with a solvent mixture of *n*-butanol - acetic acid - water (4: 1: 2). The active fractions thus obtained was concentrated and loaded on a preparative HPLC column packed with μBondapak C_{18} and eluted with a solvent mixture of acetonitrile - acetic acid - water (9: 0.2: 90.8). The active fractions were concentrated and chromatographed on Sephadex G-10 using distilled water. The active fractions finally obtained were lyophilized to give white powder of oganomycin A.

Oganomycin A(I) was obtained as colorless, hygroscopic, amorphous powder of sodium salt melting at 138~140°C with decomposition. It was easily soluble in water, but insoluble in chloroform, ether, ethyl acetate and *n*-hexane. The elemental analysis was as follows: Calcd. for $C_{24}H_{26}N_3O_{13}S_2Na \cdot 2H_2O$: C 41.88, H 4.36, N 6.11, S 9.31; Found: C 42.06, H 4.41, N 6.08, S 9.51. It showed a UV absorption maximum at 282 nm in 0.1 M phosphate buffer at pH 7.0 ($E^{1\%}_{1cm}$ 426). Its IR and PMR spectra are given in Figs. 1 and 2. It gave positive ninhydrin and negative ferric chloride reactions.

Fig. 1. IR spectrum of oganomycin A (KBr).

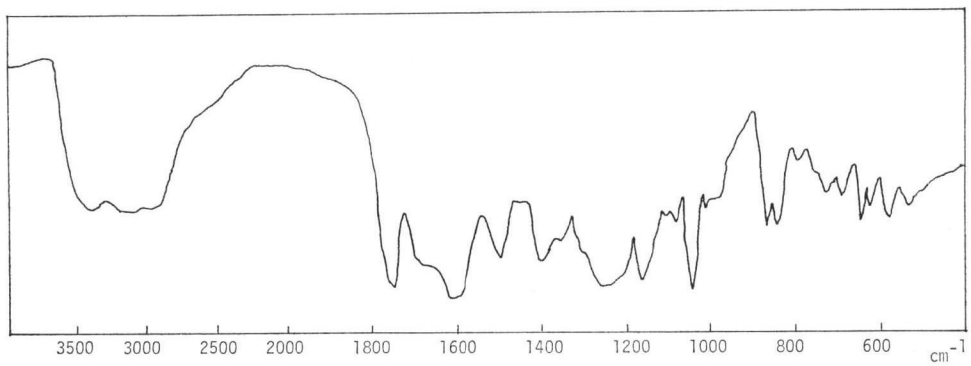


Fig. 2. ^1H NMR spectrum of oganomycin A (100 MHz in D_2O, JEOL FX100).

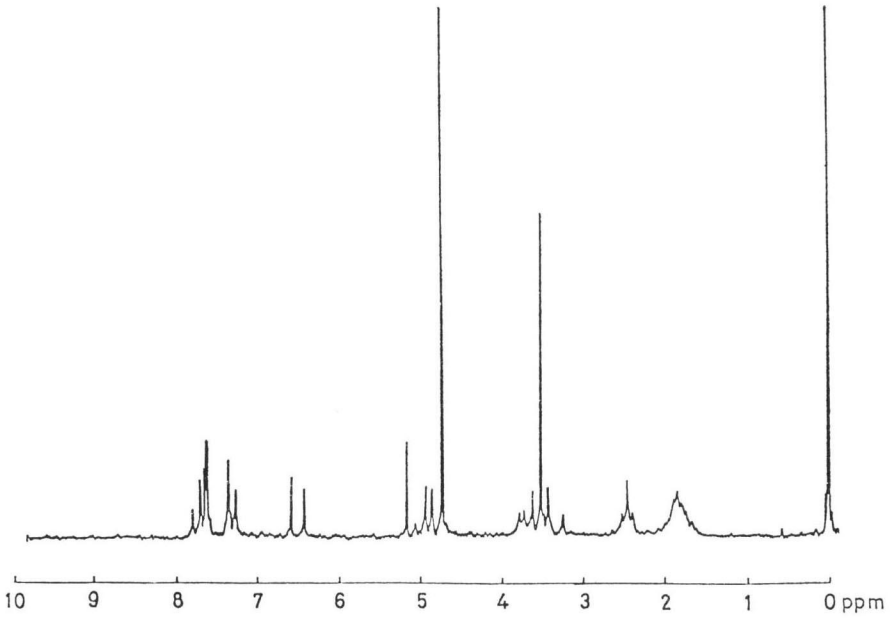
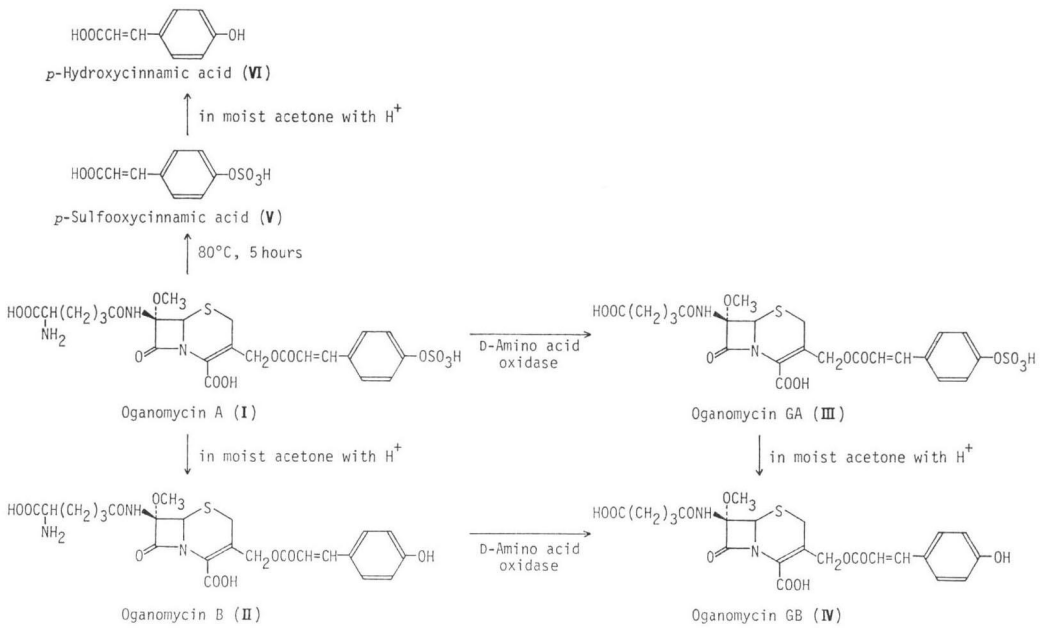


Chart 1. Oganomycin A derivatives and it's degradation products.



Oganomycin A was converted to a glutaryl derivative (oganomycin GA) using D-amino acid oxidase obtained from *Trigonopsis variabilis* IFO 0755 as described in the previous paper[1]. Oganomycin GA (III) was obtained as colorless, hygroscopic, amorphous powder which was soluble in water but insoluble in other organic solvents. The elemental analysis was as follows: Calcd. for $C_{23}H_{23}N_2O_{13}S_2Na \cdot 2\frac{1}{2}H_2O$: C 41.38, H 4.23, N 4.20, S 9.60; Found: C 41.34, H 4.10, N 4.09, S 9.46. It showed negative ninhydrin and negative ferric chloride reactions.

Oganomycins A and GA were further hydrolyzed to remove esteric sulfuric acid by treating with hydrochloric acid in moist acetone at room temperature. When purified by HPLC, oganomycins B and GB were obtained from oganomycins A and GA respectively. In the case of oganomycin GB, it was also obtained from oganomycin B by the above-stated oxidative deamination. Oganomycin B (**II**) was colorless, hygroscopic, amorphous powder, which was soluble in water but insoluble in other organic solvents. *Analysis*: Calcd. for $C_{24}H_{27}N_3O_{10}S \cdot 1\frac{1}{2}H_2O$: C 50.00, H 5.24, N 7.29, S 5.56; Found: C 49.79, H 5.13, N 7.15, S 5.42. It showed positive ninhydrin and positive ferric chloride reactions. Oganomycin GB (**IV**) was obtained as colorless, hygroscopic, amorphous powder which was soluble in water, slightly soluble in methanol and ethanol, but insoluble in other organic solvents. The analysis was as follows: Calcd. for $C_{23}H_{24}N_2O_{10}S \cdot H_2O$: C 51.30, H 4.87, N 5.20, S 5.95; Found: C 51.33, H 4.79, N 5.07, S 6.01. It showed negative ninhydrin and positive ferric chloride reactions.

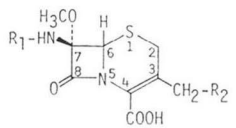
Table 1 gives PMR chemical shifts, some of the characteristic IR bands and UV absorption maxima of oganomycins A, B, GA and GB in comparison with those of cephamycin A[3~7]. Presence of β-lactam in all of the four oganomycins was suggested from the IR absorption at 1760 cm^{-1}. PMR chemical shifts of oganomycin A show close resemblance to those of cephamycin A except for absence of a single at 3.75 ppm due to the α-methoxy group of *p*-sulfooxycinnamoyl moiety. Instead, in PMR of oganomycin A, the signal for α-CH appeared as a doublet at 6.43 ppm and that for β-CH split to a doublet at 7.66 ppm. Chemical shifts of oganomycin B closely resemble those of oganomycin A, but reveal a large upfield shift by 0.38 ppm in the signals of 3′ and 5′ protons of the cinnamoyl moiety. The ferric chloride reaction changed to positive, which suggested loss of the esteric sulfuric acid in oganomycin B. The characteristic IR bands due to sulfate at 1215, 1050 and 870 cm^{-1} were further confirmed to have disappeared for oganomycin B. The chemical shifts of oganomycins GA and GB again show close resemblance to those of oganomycins A and B except for absence of the signal at 3.76 ppm (1H, t) due to 5″ methine of α-aminoadipyl moiety. Ninhydrin reaction changed to negative in these two derivatives. In consideration of the use of D-amino acid oxidase for derivation, it was concluded that these oganomycins were 7β-glutaryl compounds.

Further evidence for the structure of oganomycin A was obtained by chemical degradations. Amino acid analysis of hydrolysate of oganomycin A obtained by heating with 6 N hydrochloric acid gave α-aminoadipic acid and glycine in a molar ratio of 2: 1, which was consistent with known results on 7α-methoxycephem antibiotics[8]. To obtain the cinnamoyl side chain moiety, aqueous solution of oganomycin A was heated at 80°C for five hours, and purification was performed by HPLC and HP-20 column chromatography, which yielded colorless needles (**V**) melting at 150°C with decomposition. The crystal (**V**) was soluble in water but insoluble in other organic solvents, showed a UV absorption maximum at 268 nm in 0.1 M phosphate buffer at pH 7.0 (ε 21,600), and gave negative ferric chloride reaction. The elemental analysis was as follows: Calcd. for $C_9H_7O_6SNa \cdot \frac{1}{4}H_2O$: C 39.93, H 2.79, S 11.84; Found: C 40.05, H 2.72, S 11.60.

Crystal (**V**) was further treated with hydrochloric acid in moist acetone at room temperature to give colorless needles (**VI**) upon concentration. The crystal (**VI**) melted at 210°C, was soluble in most organic solvents but slightly soluble in cold water, showed a UV absorption maximum at 283 nm in 0 1 M phosphate buffer at pH 7.0 (ε 20,100), and gave a molecular ion at *m/z* 164 and positive ferric chloride reaction. Sulfur was not detected. When compared with an authentic sample of *p*-hydroxycinnamic acid, crystal (**VI**) gave identical PMR, IR, UV and MS spectra, which clearly indicated crystal (**VI**) was *p*-hydroxycinnamic acid and crystal (**V**) was sodium salt of *p*-sulfooxycinnamic acid. Thus structure of oganomycin A and its derivatives were elucidated as given in Chart 1.

Organomycins A and B were found to be much more stable than cephamycins A and B. Fig. 3 shows degradation of these cephem compounds in 0.1 M phosphate buffer at pH 7.5 at 29°C. In the course of 24 hours, degradation of oganomycins A and B was less than 20 %, while that of cephamycins A and B was more than 70 %. Degradation constants (*Kd*) of oganomysins A and B were 0.83×10^{-2} and 0.53×10^{-2} $hour^{-1}$ and those of cephamycins A and B were 7.25×10^{-2} and 4.96×10^{-2} $hour^{-1}$ respectively.

Table 1. Physical properties of oganomycins and cephamycins.



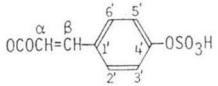
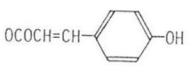
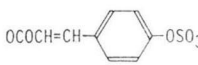
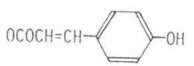
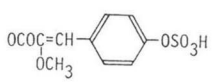
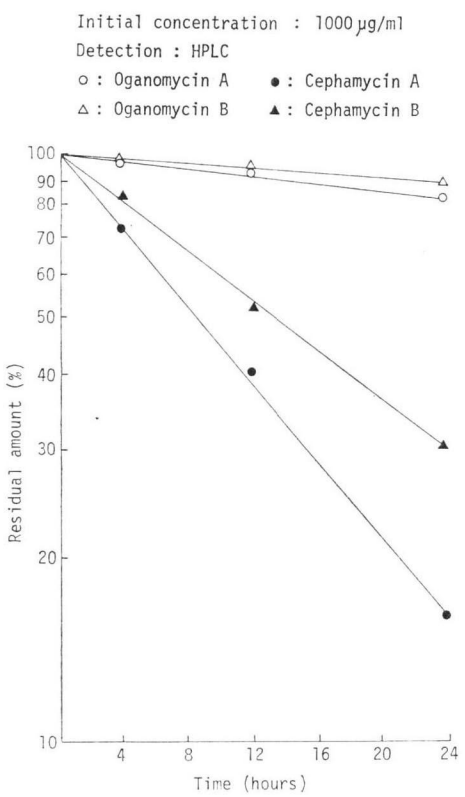
		Oganomycin A	Oganomycin B	Oganomycin GA	Oganomycin GB	Cephamycin A
	R_1	HO_2C-CH-$(CH_2)_2$-CH_2CO (5″ 3″ 4″ 2″ 1″), NH_2	HO_2C-CH-$(CH_2)_2$-CH_2CO, NH_2	HO_2C-$(CH_2)_2$-CH_2CO	HO_2C-$(CH_2)_2$-CH_2CO	HO_2C-CH-$(CH_2)_2$-CH_2CO, NH_2
	R_2					
PMR (δ, ppm; D_2O)	3″, 4″-CH_2CH_2	1.90, 4H, m	1.88, 4H, m	1.95, 4H, m	1.94, 4H, m	1.87, 4H, m
	2″-CH_2	2.49, 2H, t	2.50, 2H, t	2.44, 2H, t	2.44, 2H, t	2.49, 2H, t
	5″-CH	3.76, 1H, t	3.76, 1H, t	—	—	3.90, 1H, t
	2-CH_2	3.34～3.67, 2H dd, J=18Hz	3.35～3.73, 2H dd, J=18Hz	3.36～3.73, 2H dd, J=18Hz	3.36～3.74, 2H dd, J=18Hz	3.35～3.78, 2H dd, J=18Hz
	3-CH_2	5.00, 2H, dd J=13Hz	4.92, 2H, dd J=13Hz	4.93, 2H, dd J=13Hz	4.92, 2H, dd J=13Hz	5.01, 2H, dd J=13Hz
	6-CH	5.16, 1H, s	5.19, 1H, s	5.19, 1H, s	5.19, 1H, s	5.21, 1H, s
	7-OCH_3	3.53, 3H, s	3.54, 3H, s	3.55, 3H, s	3.54, 3H, s	3.55, 3H, s
	α-OCH_3	—	—	—	—	3.75, 3H, s
	α-CH	6.43, 1H, d J=16Hz	6.41, 1H, d J=16Hz	6.54, 1H, d J=16Hz	6.40, 1H, d J=16Hz	—
	β-CH	7.66, 1H, d J=16Hz	7.71, 1H, d J=16Hz	7.55, 1H, d J=16Hz	7.71, 1H, d J=16Hz	7.17, 1H, s
	3′, 5′-CH	7.32, 2H, d J=8.5Hz	6.94, 2H, d J=8.5Hz	7.35, 2H, d J=8.5Hz	6.93, 2H, d J=8.5Hz	7.35, 2H, d J=8.5Hz
	2′, 6′-CH	7.61, 2H, d J=8.5Hz	7.59, 2H, d J=8.5Hz	7.71, 2H, d J=8.5Hz	7.58, 2H, d J=8.5Hz	7.83, 2H, d J=8.5Hz
IR	1760 (β-lactam)	+	+	+	+	+
(KBr, cm^{-1})	870, 1050, 1215 (SO_3H)	+	−	+	−	+
UV $\lambda_{max}^{H_2O}$ nm (pH 7.0)		282	308	282	307	285

Fig. 3. Stability of oganomycins and cephamycins at 29°C in 0.1 M phosphate buffer (pH 7.5).



Structural difference of oganomycins A and B from cephamycins A and B exists in the absence of the methoxy group attached to the α-carbon of cinnamoyl side chains. *Ka* values of these cinnamic acid derivatives of the side chains were 2.00×10^{-5} and 1.41×10^{-5} for oganomycins A and B and 15.8×10^{-5} and 12.5×10^{-5} for cephamycins A and B respectively. As shown in Fig. 4, the *Kd* value of each cephem compound corresponds linearly to the *Kd* value of each acid of the side chain moiety. Owing to the inductive effect of the α-methoxy group, cephamycins A and B are considered to be more liable to hydrolysis of the cinnamoyl ester linkage.

Oganomycins A and B showed antibacterial activities against Gram-positive and Gram-negative bacteria. Some of representative results are given in Table 2 in comparison with those of cephamycins A and B.

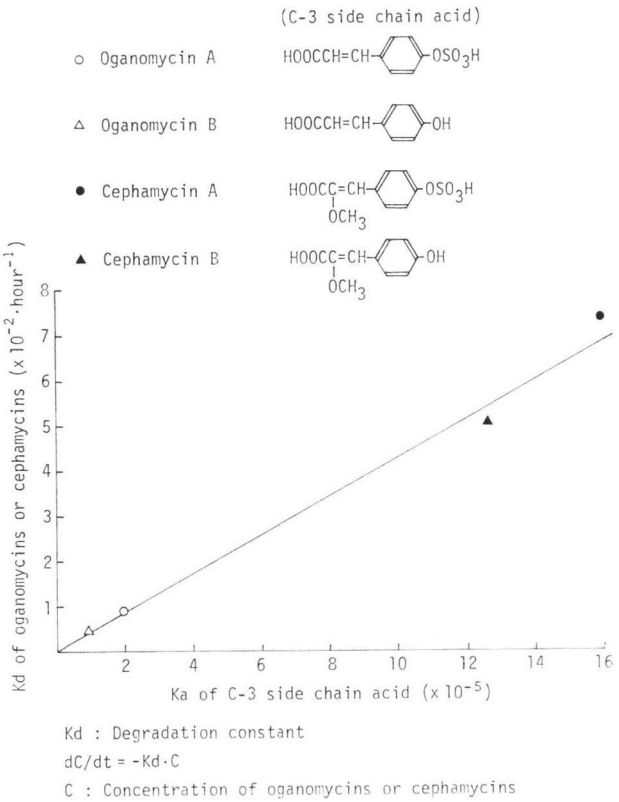
As reported in the previous paper[1], we have successfully obtained 7-methoxycephem compounds having heterocyclic thiomethyl side chains at the 3 position by direct fermentation. But this method is not applicable when we intend to obtain a series of 7-methoxycephalosporin derivatives other than 3-heterocyclic thiomethyl ones. Oganomycin A and its hydrophobic derivative, oganomycin GB, are of particular interest as alternative intermediates. As shown in Fig. 3, oganomycins A and B are much more stable than cephamycins A and B. Thus oganomycin A is expected to be accumulated at a high concentration in the broth, and to be recovered and purified much more easily than cephamycin A or B on an industrial scale.

Table 2. Antibacterial spectra of oganomycins and cephamycins.

Test organism	MIC (μg/ml)*					
	Oganomycin A	Oganomycin B	Oganomycin GA	Oganomycin GB	Cephamycin A	Cephamycin B
Bacillus subtilis ATCC 6633	12.5	3.13	100	100	50	6.25
Staphylococcus aureus ATCC 6538P	100	50	100	50	>100	100
Escherichia coli NIHJ	6.25	3.13	6.25	6.25	25	12.5
Klebsiella pneumoniae ATCC 10031	25	3.13	25	12.5	50	25
Salmonella typhi H901W	3.13	12.5	50	25	25	50
Salmonella enteritidis 1891	6.25	1.56	50	25	25	12.5
Shigella boydii IID 627	6.25	6.25	25	12.5	25	50
Proteus mirabilis IFM OM-9	0.78	0.78	12.5	6.25	6.25	6.25
Proteus vulgaris OXK US	0.39	0.78	6.25	3.13	1.56	6.25
Pseudomonas aeruginosa NCTC 10490	>100	>100	>100	>100	>100	>100

* Determined on heart infusion agar inoculated with one loopful of overnight culture.

Fig. 4. Influence of *Ka*'s of C-3 side chain acids on degradation of oganomycins and cephamycins.



Kd : Degradation constant

$dC/dt = -Kd \cdot C$

C : Concentration of oganomycins or cephamycins

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