Communications to the editor

OGANOMYCINS, NEW 7-METHOXY-CEPHALOSPORINS PRODUCED BY PRECURSOR FERMENTATION WITH HETEROCYCLIC THIOLS

Sir:

In the search for novel methoxycephalosporins, we encountered new, 3-substituted 7-methoxycephalosporins when heterocyclic thiols (*cf.* RSH's in Table 1) were added to fermenting broth of an actinomycete, *Streptomyces oganonensis* n. sp. strain Y-G19Z, which was isolated from a soil sample collected at Ogano Town in Chichibu, Saitama Prefecture, Japan, and was shown by us to produce methoxycephalosporins.[1] The new, methoxycephalosporins were named oganomycins.

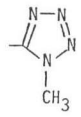
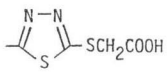
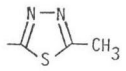
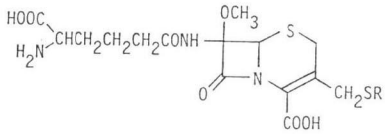
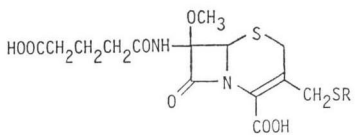
Oganomycin G was produced as a main antibiotic when Y-G19Z strain was grown at 30°C in a medium adjusted to pH 7.0 which contained 7% starch, 2% gluten meal, 2% soybean meal, 0.8% glycerol, 0.1% Casamino acid, 0.01% ferric sulfate and 0.2% of a precursor (5-mercapto-1-methyl-1H-tetrazole). After 4~5 days when the potency of oganomycin G, assayed by the paper disk-plate method against *Proteus mirabilis* IMF-OM9, reached a maximum, the broth was harvested and filtered. Oganomycin G in the filtrate was adsorbed on Amberlite XAD-2 at pH 3.0 and eluted with 50% acetone. The eluate was concentrated under reduced pressure. The antibiotic in it was adsorbed on Amberlite IRA-68 (Cl^-) and eluted with 1 M sodium nitrate solution at pH 7.2 containing 0.1 M sodium acetate. After repetition of XAD-2 adsorption and acetone elution as above-stated, the antibiotic was chromatographed on DEAE-Sephadex A-25 (CH_3COO^-) equilibrated and eluted with 0.5 M ammonium bromide solution (adjusted to pH 3.1 with acetic acid). Active fractions, after XAD-2 adsorption and acetone elution, were further chromatographed on a cellulose powder (Avicel) using successively three different solvent systems, *i.e.* isopropanol - water (7: 3), isopropanol - *n*-butanol - acetic acid - water (21: 3: 7: 9) and *n*-butanol - acetic acid - water (4: 1: 2). Active fractions showing a single specific spot on an Avicel thin-layer chromatogram were finally chromatographed on Sephadex G-10 with distilled water. Active fractions thus obtained were lyophilized to give white powder of oganomycin G which was found to show a single peak on a high performance liquid chromatogram.

Oganomycin G was obtained as an amphoteric, hygroscopic, colorless amorphous powder melting at 160~170°C with decomposition. It was easily soluble in water, sparingly soluble in methanol, but insoluble in chloroform, ether, ethyl acetate and *n*-hexane. It was stable at pH 1.5~8.0 in aqueous solution. The elemental analysis was as follows: calcd. for $C_{17}H_{22}N_7O_7S_2$-$Na \cdot 2H_2O$: C 36.49, H 4.68, N 17.52, S 11.46; found: C 37.48, H 4.25, N 16.74, S 10.90. It showed a maximum in UV spectrum at 273 nm in 0.01 M phosphate buffer at pH 6.4 ($E^{1\%}_{1cm}$ 204). The IR spectrum is presented in Fig. 1. The PMR chemical shifts are given in Table 1. It gave a positive ninhydrin reaction.

For further purification, oganomycin G was converted to glutaryl derivative (organomycin GG) by oxidative deamination at its α-amino-adipyl moiety using D-amino acid oxidase from *Trigonopsis variabilis* IFO-0755[2]. Oganomycin G was treated with Triton X 100-activated *Trigonopsis variabilis* cells at 33°C under aeration in a phosphate buffer at pH 8.1 containing 0.026% sodium azide and adequate amount of hydrogen peroxide. After the ninhydrin test changed to negative, the reaction mixture was centrifuged at 4°C. Oganomycin GG in the supernatant was extracted repeatedly with ethyl acetate at pH 1.5~2.0. The extracted antibiotic was transferred to phosphate buffer at pH 6.0, retransferred to ethyl acetate: and, after removal of the solvent, chromatographed on Avicel with *n*-butanol - acetic acid - water mixture (4: 1: 2). Active fractions were lyophilized to give white powder of pure oganomycin GG, which gave needle crystals on recrystallization from methyl ethyl ketone.

Oganomycin GG was obtained as acidic, colorless needles melting at 163.5°C with decomposition. It was easily soluble in methanol and ethanol; soluble in water, isopropanol, *n*-butanol, benzyl alcohol, ethyl acetate, methyl ethyl ketone

Table 1. Structures and PMR chemical shifts of oganomycins.

R of precursor RSH				
Structure				
Chemical shift (δ, ppm; D_2O)	Oganomycin G	Oganomycin F	Oganomycin H	Oganomycin I
3′, 4′-CH_2CH_2	2.36, 4H, m	2.35, 4H, m	2.34, 4H, m	2.30, 4H, m
2′-CH_2	2.96, 2H, m	2.96, 2H, m	2.95, 2H, m	2.93, 2H, m
5′-CH	4.38, 1H, m	4.25, 1H, m	4.26, 1H, m	4.26, 1H, m
2 -CH_2	3.83~4.34, 2H dd, J=18 Hz	3.73~4.33, 2H dd, J=18 Hz	3.72~4.31, 2H dd, J=18 Hz	3.69~4.29, 2H dd, J=18 Hz
3 -CH_2	4.47~4.83, 2H dd, J=14 Hz	4.42~4.91, 2H dd, J=14 Hz	4.40~4.95, 2H dd, J=14 Hz	4.45~4.99, 2H dd, J=14 Hz
6 -CH	5.59, 1H, s	5.63, 1H, s	5.63, 1H, s	5.56, 1H, s
7 -OCH_3	3.98, 3H, s	4.00, 3H, s	3.99, 3H, s	3.97, 3H, s
Heterocyclic proton	4.50, 3H, s	4.44, 2H, s	3.19, 3H, s	9.85, 1H, s
Structure				
Chemical shift (δ, ppm; CD_3OD)	Oganomycin GG	Oganomycin GF	Oganomycin GH	Oganomycin GI
3′-CH_2	1.94, 2H, m	1.93, 2H, m	1.92, 2H, m	1.92, 2H, m
2′, 4′-CH_2CH_2	2.40, 4H, m	2.41, 4H, m	2.40, 4H, m	2.40, 4H, m
2 -CH_2	3.40~3.83, 2H dd, J=18 Hz	3.37~3.81, 2H dd, J=18 Hz	3.38~3.80, 2H dd, J=18 Hz	3.39~3.85, 2H dd, J=18 Hz
3 -CH_2	4.17~4.50, 2H dd, J=14 Hz	4.15~4.63, 2H dd, J=14 Hz	4.17~4.64, 2H dd, J=14 Hz	4.24~4.73, 2H dd, J=14 Hz
6 -CH	5.02, 1H, s	5.05, 1H, s	5.03, 1H, s	5.03, 1H, s
7 -OCH_3	3.51, 3H, s	3.51, 3H, s	3.51, 3H, s	3.51, 3H, s
Heterocyclic proton	3.99, 3H, s	4.11, 2H, s	2.71, 3H, s	9.35, 1H, s

and methyl propyl ketone; but insoluble in chloroform, ether, benzene and *n*-hexane. It was stable at pH 1.5~8.0 in aqueous solution. It gave following analysis: calcd. for $C_{16}H_{20}N_6O_7S_2$: C 40.67, H 4.27, N 17.79, S 13.57; found: C 40.80, H 4.27, N 17.59, S 13.29. It gave a UV absorption maximum at 269 nm in 0.01 M phosphate buffer at pH 6.5 (ε 11,100). Its IR spectrum is given in Fig. 2. The PMR chemical shifts are shown in Table 1. It gave a negative ninhydrin reaction.

Oganomycin G gave α-aminoadipic acid after 6 N hydrochloric acid hydrolysis, and 5-mercapto-1-methyl-1H-tetrazole (*m/e* 116), the precursor

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

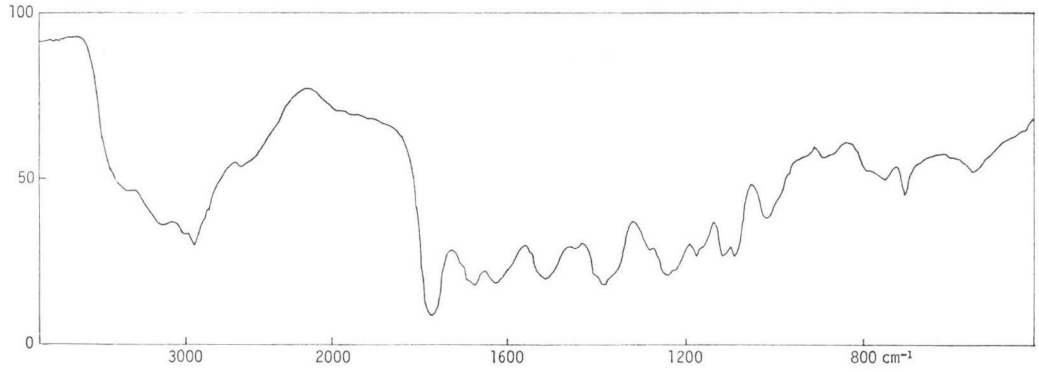
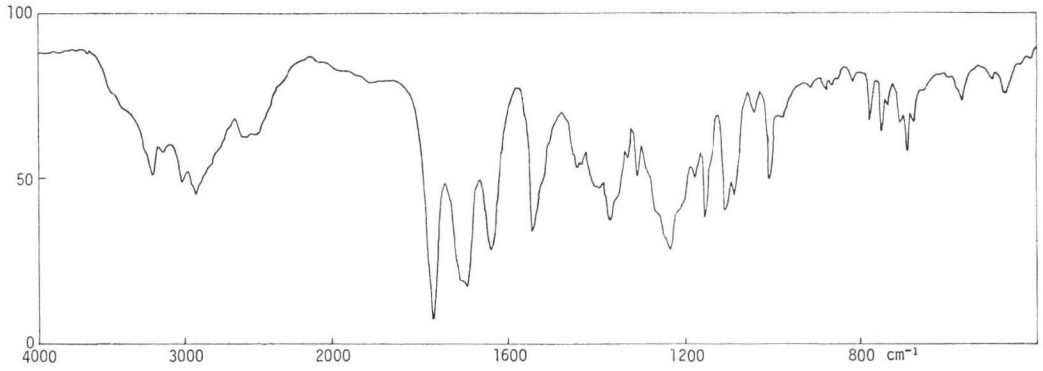


Fig. 2. IR spectrum of oganomycin GG (KBr).



moiety, after Dowex 50W (H^+) hydrolysis in methanol. From these results and those indicating the presence of 7-methoxy cephem skeleton (IR absorption at 1765 cm^{-1} due to β-lactam; PMR signals (δ, ppm) in D_2O at 3.83~4.34 (2H, dd, J=18 Hz), 5.59 (1H, s) and 3.98 (3H, s) and also from a PMR signal at 4.50 (3H, s) which indicated N-methyl protons of tetrazole moiety, the structure given in Table 1 was postulated for oganomycin G. Oganomycin GG gave glutaric acid (*m/e* 276 after trimethylsilylation) instead of α-aminoadipic acid after hydrochloric acid hydrolysis. Further, from the IR absorption bands and the PMR signals presented in Table 1 and from the elemental analysis, the structure of oganomycin GG was assigned as given in Table 1. A ^{13}C-NMR study supported this structure. Details will be reported elsewhere.[8]

By similar procedures, we obtained oganomycins F, H, I and GF, GH and GI as illustrated in Table 1. When disulfides, RSSR's, were used instead of RSH's in Table 1, the same 3-substituted derivatives were obtained.

Oganomycin F, oganomycin H and oganomycin I were obtained as amphoteric, hygroscopic, colorless amorphous powders showing solubilities and stabilities similar to those of oganomycin G and giving a positive ninhydrin reaction. They gave similar IR spectra, and liberated α-aminoadipic acid and precursor moieties after hydrolysis. Their chemical shifts are given in Table 1. Oganomycin F showed: m.p. at 156~170°C (dec.); λ_{max} at 287 nm in phosphate buffer at pH 6.4 ($E^{1\%}_{1cm}$ 240); analysis calcd. for $C_{19}H_{21}N_5O_9S_4Na_2$: C 35.79, H 3.32, N 10.98, S 20.11; found: C 35.95, H 3.87, N 10.85, S 18.33. Oganomycin H showed: m.p. at 170°C (dec.); λ_{max} at 272 nm at pH 6.4; analysis calcd. for $C_{18}H_{22}N_5O_7S_3Na \cdot 2H_2O$: C 37.56, H 4.55, N 12.17, S 16.71; found: C 37.82, H 4.01, N 12.90, S 14.97. Oganomycin I showed: m.p. at 175~180°C (dec.); λ_{max} at 274 nm at pH 6.4 ($E^{1\%}_{1cm}$ 232); analysis calcd. for $C_{17}H_{21}N_5O_7S_3 \cdot 2H_2O$:

Table 2. Antibacterial spectra of oganomycins.

Test organism	MIC (μg/ml)*		
	Oganomycin G	Oganomycin H	Oganomycin I
Bacillus megaterium 10778	6.25	12.5	12.5
Bacillus subtills ATCC 6633	6.25	12.5	6.25
Staphylococcus aureus Smith	100	50	100
Staphylococcus aureus Terashima	50	25	50
Staphylococcus aureus Onuma	50	50	100
Micrococcus flavus	1.56	3.13	1.56
Sarcina lutea	6.25	25	6.25
Corynebacterium xerosis	6.25	12.5	6.25
Mycobacterium 607	50	50	100
Mycobacterium phlei	50	50	100
Escherichia coli O-1	3.13	6.25	3.13
Escherichia coli NIHJ	3.13	6.25	6.25
Klebsiella pneumoniae ATCC 10031	3.13	3.13	6.25
Vibrio HY 133	6.25	12.5	6.25
Salmonella cholerae-suis 1348	1.56	3.13	1.56
Salmonella typhi H901W	3.13	3.13	3.13
Salmonella enteritidis 1891	1.56	1.56	1.56
Shigella flexneri 2a 1675	3.13	6.25	6.25
Shigella sonnei II 37148	3.13	6.25	6.25
Proteus vulgaris OXK US	1.56	3.13	3.13
Proteus mirabilis IFM-OM9	1.56	3.13	3.13
Pseudomonas aeruginosa ATCC 8689	100	50	100
Pseudomonas ovalis IAM 1002	100	50	100
Pseudomonas melanogenum	100	50	100
Pseudomonas aeruginosa 99 (GM-R)	100	50	100

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

MIC's of cephamycin C are reported as follows (in μg/ml): *Staphylococcus aureus* 800; *Streptococcus agalactiae* 400; *Streptococcus pyogenes* 200; *Diplococcus pneumoniae* 200; *Escherichia coli* 13~50; *Klebsiella pneumoniae* 6~25; *Aerobacter aerogenes* 100~>800; *Salmonella pullorum* 13; *Salmonella schottmuelleri* 25; *Shigella* 13; *Proteus mirabilis* 0.4~6; *Proteus morganii* 13; *Proteus vulgaris* 3~13; *Pseudomonas aeruginosa* >800. (Antimicr. Agents & Chemoth. 2: 281~286, 1972).

C 37.84, H 4.67, N 12.98, S 17.83; found: C 37.53, H 4.36, N 12.77, S 16.42.

Oganomycins GF, GH and GI were obtained as acidic, colorless amorphous powders showing solubilities and stabilities similar to oganomycin GG and giving negative ninhydrin reactions. They gave PMR signals presented in Table 1 and had IR spectra similar to oganomycin GG. They gave glutaric acid after hydrochloric acid hydrolysis. Oganomycin GF showed: m.p. at 75~78°C; λ_{max} at 278 nm at pH 6.5 (ε 13,800); analysis calcd. for $C_{18}H_{20}N_4O_9S_4 \cdot 2H_2O$: C 35.99, H 4.03, N 9.33; found: C 35.77, H 3.81, N 9.42. Oganomycin GH showed: m.p. at 95~99°C; λ_{max} at 273 nm at pH 6.5 (ε 11,300); analysis calcd. for $C_{17}H_{20}N_4O_7S_3$: C 41.79, H 4.13, N 11.47; found: C 41.89, H 4.27, N 11.17. Oganomycin GI showed: m.p. at 88~92°C, λ_{max} at 274 nm at pH 6.5 (ε 13,000); analysis calcd. for $C_{16}H_{18}N_4O_7S_3 \cdot 1/2H_2O$: C 39.74, H 3.96, N 11.59; found: C 39.69, H 3.87, N 11.32.

Oganomycins showed the following Rf's on silica gel thin-layer chromatogram developed with chloroform - methanol - formic acid mixture (80: 20: 2): G 0.02, F 0.00, H 0.02, I 0.02, GG 0.49, GF 0.35, GH 0.59, GI 0.49; on Avicel SF with *n*-butanol - acetic acid- water (4: 1: 2): G 0.34, F 0.37, H 0.44, I 0.36, GG 0.64, GF 0.72, GH 0.77 and GI 0.65. Oganomycins gave the following retention times on a high performance

liquid chromatography using μ Bondapak C_{18} (Waters Co.) and solvent mixture of acetonitrile - water (10: 90) adjusted to pH 3.3 with acetic acid (′ and ″ mean minute and second): G 1′53″, F 3′14″, H 2′55″, I 1′56″, GG 4′54″, GF 13′24″, GH 11′18″ and GI 5′28″.

Oganomycins showed antibacterial activities against Gram-positive and -negative bacteria. MIC's of oganomycins G, H and I are given in Table 2. Oganomycins GG, GH and GI were about 1/2 to 1/8 as active as their parent compounds.

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2) Senthe Shanmuganathan, S. & W. J. Nickerson: Transaminase and D-amino acid oxidase of *Trigonopsis variabilis*. J. Gen. Microbiol. 27: 465～471, 1962
3) Watanabe, S.; T. Saito, H. Gushima, K. Murakami, Y. Oka & T. Osono: Oganomycins, new 3-heterocyclothiomethyl-7-methoxycephalosporins (Y-G19Z's) produced by precursor fermentation by a *Streptomyces* strain. I. Fermentation, isolation, purification, oxidative deamination and physicochemical properties of oganomycins G and GG. J. Antibiotics, in preparation