# MUREIDOMYCINS E AND F, MINOR COMPONENTS OF MUREIDOMYCINS

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Mureidomycins (MRDs) E and F were isolated from a culture filtrate of *Streptomyces flavidovirens* SANK 60486 which produces MRDs A~D. They possessed the same molecular formulae, $C_{39}H_{48}N_8O_{12}S$ and very similar UV, IR and NMR spectra, but differed clearly from each other in HPLC profile. From the hydrolysates of MRDs E and F, 8-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid and 6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid were detected, respectively, which were not detected from those of MRDs A~D. They showed strong anti-pseudomonal activity but less active than MRD A. MRDs E and F were synthesized from MRD A and formaldehyde through Pictet-Spengler reaction.

Mureidomycins (MRDs) A~D are peptidylnucleoside antibiotics, selectively active against *Pseudomonas aeruginosa*[1~4]. They inhibit bacterial peptidoglycan synthesis through translocase[5]. One of their characteristics is that among the known inhibitors of translocase, they do not inhibit other lipid-intermediate formation than undecaprenyl pyrophosphoryl *N*-acetylmuramyl-pentapeptide, the first intermediate of the lipid cycle of bacterial peptidoglycan synthesis[6], though other inhibitors such as tunicamycin[7,8] and amphomycin[9,10] inhibit both bacterial and mammalian lipid-intermediate formation. In this paper, we mention about the isolation, physico-chemical properties, structures, biological properties and synthesis from MRD A of new minor components, MRDs E and F[11].

## Materials and Methods

### Fermentation of MRDs E and F

*Streptomyces flavidovirens* SANK 60486 was cultured as described previously[1].

### Isolation of MRDs E and F

Thirty liters of the fermentation broth was filtered with the aid of Celite 545 and the cake was washed with water. Thirty liters of the filtrate thus obtained was adsorbed on a column of Amberlite XAD-2 (3 liters) and after washing with 15 liters of water and 15% of aqueous methanol successively, the antibiotics were eluted with 15 liters of 40% aqueous methanol. The active eluate was concentrated *in vacuo* and lyophilized to obtain 17.4 g of the crude powder. A 17-g aliquot of this crude powder was dissolved in 3 liters of distilled water and adsorbed on a column of Amberlite CG-50 ($H^+$ type, 800 ml). The active fractions eluted with 0.5 N $NH_4OH$ were collected and concentrated to 1 liter under reduced pressure. The concentrate was adsorbed on a column of Whatman DE-52 (1 liter) pre-equilibrated with 0.1 M $NH_4HCO_3$ and eluted with 0.2 M $NH_4HCO_3$. The active fractions (1,000 ml) were collected and adsorbed on a column containing 200 ml of Diaion HP-20 resin, and the column was eluted with 500 ml of 50% aqueous acetone, to give active components. The fractions containing the active components were concentrated and lyophylized. Then this powder was dissolved in 500 ml of water and adsorbed on a column containing 500 ml of DE-52 pre-equilibrated with 0.05 M $NH_4HCO_3$. The column was then washed with 0.05 M $NH_4HCO_3$ and eluted with 0.1 M $NH_4HCO_3$, to give fractions, each containing 20 ml of the eluent. The fractions from

80 to 130 were collected and de-salted by HP-20 to afford 3.1 g of the powder. 3.0 g of this powder was loaded on a column containing 100 g of silica gel and the column was eluted with the mixture of *n*-butanol, *n*-propanol and water (8:4:1), to give fractions, each containing 20 ml of the eluent. The fractions from 13 to 70 were collected and lyophylized to give 320 mg of the powder containing MRDs A, E and F. 300 mg of this powder was developed with 30% aqueous MeOH through a column containing 1,000 ml of Toyopearl HW-40 and the eluent was collected in 10 ml each. The fractions from 95 to 105 were collected and adsorbed with 10 ml of CG-50 ($H^+$ type) and then eluted with 0.5 M $NH_4OH$. The active fractions were collected and lyophylized. Thus 15 mg of MRD E was obtained. From fraction numbers 80 to 90, 32 mg of MRD F was obtained.

Isolation of Tetrahydroisoquinoline Derivatives from Acid Hydrolysates of MRDs E and F

A 30-mg aliquot of MRDs E or F was hydrolyzed in conc. HCl - AcOH (1:1) for 20 hours at 105°C in a sealed tube. After evaporation of the solvent under reduced pressure, the residue was chromatographed on Toyopearl column using the upper layer of *n*-BuOH - AcOH - $H_2O$ (4:1:5) as eluent. Each fraction was checked by silica gel TLC (solvent; *n*-BuOH - AcOH - $H_2O$ = 4:1:2, ninhydrin). Fractions of Rf value 0.5(I) or 0.45(II) were collected and further purified by HPLC (ODS-H-5251; Senshu Co., 8 ml/minute, 12.5% acetonitrile - 0.2% TFA). The $^1H$ NMR data for I and II were as follows: I ($D_2O$), $\delta$ 2.93 (1H, dd, $J$ = 11.4, 17.2 Hz), 3.18 (1H, dd, $J$ = 4.9, 17.2 Hz), 3.82 (1H, dd, $J$ = 4.9, 11.4 Hz), 4.01 (1H, d, $J$ = 16.3 Hz), 4.30 (1H, d, $J$ = 16.3 Hz), 6.64 (1H, d), 6.69 (1H, d), 7.04 (1H, t); II ($D_2O$), $\delta$ 2.94 (1H, dd, $J$ = 11.0, 17.2 Hz), 3.17 (1H, dd, $J$ = 5.5, 17.2 Hz), 3.91 (1H, dd, $J$ = 5.5, 11.0 Hz), 4.13 (1H, d, $J$ = 15.4 Hz), 4.19 (1H, d, $J$ = 15.4 Hz), 6.65 (2H), 6.96 (1H, d).

Synthesis of MRDs E and F

270 mg of MRD A was dissolved in 60 ml of water, and then 300 $\mu$l of 30% aqueous formaldehyde[12] was added to it and the mixture was kept at room temperature overnight. Then the mixture was developed through 1,500 ml of Toyopearl HW-40 with 30% aqueous methanol. The eluent was collected at 15 ml each. From fraction numbers 81 to 88 and from 92 to 100, 65 mg of MRD F and 64 mg of MRD E were obtained, respectively.

## Results and Discussion

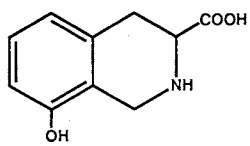
### Physico-Chemical Properties of MRDs E and F

They are amphoteric white powders, soluble in water. Their molecular weights and molecular formulae (in parenthesis) were determined to be both 852 ($C_{39}H_{48}N_8O_{12}S$) by mass spectrometry and elemental analyses. By acid hydrolysis, they produced uracil, *m*-tyrosine, and 2-amino-*N*-3-methylaminobutyric acid in common. 8-Hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (**I**) and 6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (**II**) (Fig. 1) were obtained from MRDs E and F, respectively. The total physico-chemical properties of MRDs E and F are summarized in Table 1.

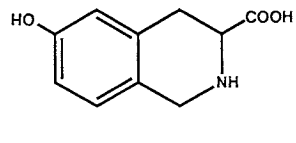
### Structural Elucidation

The high resolution mass measurements of the protonated molecular ions from positive ion FAB-MS

Fig. 1. Structures of tetrahydroisoquinoline derivatives obtained from MRDs E and F.




8-Hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid
(**I**)



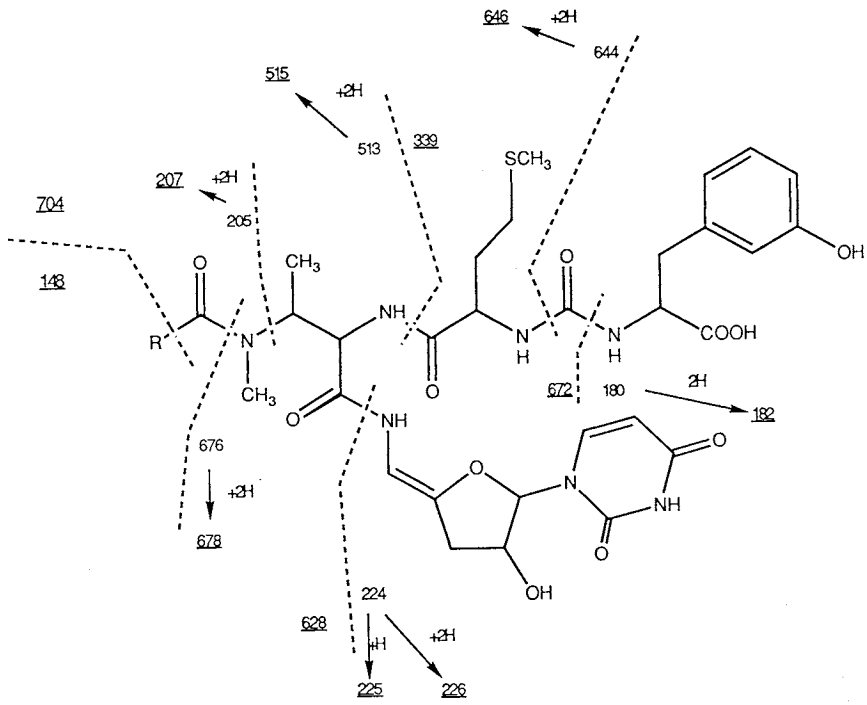

6-Hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid
(**II**)

Table 1. Physico-chemical properties of MRDs E and F.

| | MRD E | MRD F |
|---|---|---|
| Appearance | Amphoteric white powder | Amphoteric white powder |
| Molecular formulae | $C_{39}H_{48}N_8O_{12}S$ | $C_{39}H_{48}N_8O_{12}S$ |
| Molecular weight | 852 | 852 |
| Specific rotation | −34.2° (*c* 1.17, 50% aq MeOH) | −40.3° (*c* 1.05, 50% aq MeOH) |
| UV $\lambda_{max}$ nm ($E^{1\%}_{1cm}$) | | |
| pH 7.0 ($H_2O$) | 258 (252) | 258 (232) |
| pH 2.0 ($H_2O$) | 258 (247) | 258 (232) |
| pH 9.0 ($H_2O$) | 240 (432), 265 (235 sh), 295 (80 sh) | 240 (352), 265 (200 sh), 295 (64 sh) |
| Solubility | | |
| Soluble: | Water, MeOH | Water, MeOH |
| Insoluble: | EtOAc, $CHCl_3$, benzene | EtOAc, $CHCl_3$, benzene |
| Color reaction | Ninhydrin, $H_2SO_4$, iodine, ferric chloride, Baeyer reaction | Ninhydrin, $H_2SO_4$, iodine, ferric chloride, Baeyer reaction |
| TLC[a] | | |
| Rf | 0.39 | 0.34 |
| HPLC[b] | | |
| Retention time | 4.7 minutes | 5.3 minutes |

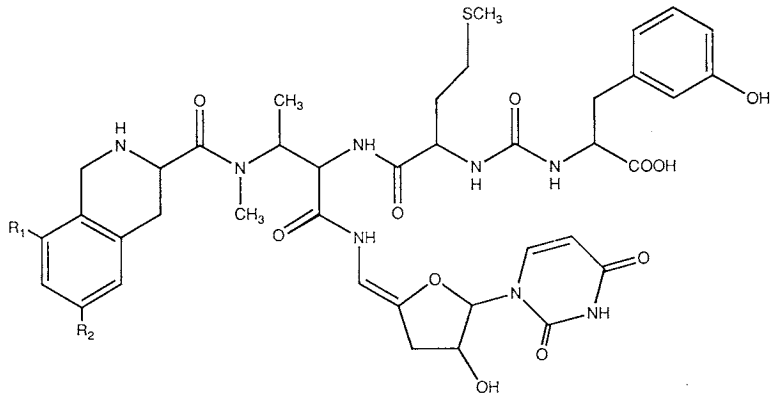
[a] Kieselgel 60 $F_{254}$, *n*-butanol - *n*-propanol - water (4 : 2 : 1).
[b] Aquasil SS 372-N (Senshu Kagaku Co.), chloroform - 2-propanol - MeOH - water (200 : 100 : 100 : 40), 1.0 ml/minute, 260 nm.

Fig. 2. MS/MS fragmentations of MRDs E and F (R = $C_9H_{10}NO$).




of MRDs E and F were recorded as 853.3212 and 853.3180, respectively, which both correspond to $C_{39}H_{49}N_8O_{12}S$ (calcd 853.3165; M+H). Product ions by MS/MS measurements of $(M+H)^+$ ions from the FAB-MS of MRDs E and F were indicative of the *N*-terminus substitution for one of *m*-tyrosines in MRD A as shown in Fig. 2. These ions, which were 12 amu bigger than analogous ions produced from MRD

Fig. 3. Structures of MRDs E and F.



| | $R_1$ | $R_2$ |
|---|---|---|
| Mureidomycin E | OH | H |
| F | H | OH |

A, were *m/z* 672, 646, 628, 515, 207, and 148. The fragment ions at *m/z* 704, 678, 339, 226, 225, and 182 were seen in the all MS/MS spectra of MRDs E, F, and A as they are unaffected by the different substitutions. These data suggest that MRDs E and F are isomeric compounds to each other. The NMR data also indicates MRDs E and F to be similar to MRD A except that there are new signals in the NMR spectra of MRDs E and F instead of one of the *m*-tyrosine residues in MRD A. The MS and NMR data indicates that these new signals correspond to isoquinoline structures, which is proven by obtaining isoquinoline derivatives (see Fig. 1) from hydrolysis of MRDs E and F. From these results, the total structures of MRDs E and F were determined as shown in Fig. 3.

Table 2. Antimicrobial activity of MRDs E and F.

| Test organism | MIC (μg/ml) | |
|---|---|---|
| | MRD E | MRD F |
| *Staphylococcus aureus* FDA 209P JC-1 | >200 | >200 |
| *Escherichia coli* NIHJ JC-2 | >200 | >200 |
| *Proteus mirabilis* SANK 70461 | >200 | >200 |
| *Pseudomonas aeruginosa* SANK 75775 | 6.25 | 25 |
| *P. aeruginosa* SANK 75115 | 25 | 50 |
| *P. aeruginosa* SANK 70970 | 25 | 100 |
| *P. aeruginosa* NRRL B1000 | 25 | 3.13 |
| *P. aeruginosa* ATCC 13388 | 25 | 3.13 |
| *P. aeruginosa* SANK 70579 | <0.4 | 1.56 |
| *P. aeruginosa* NCTC 10490 | <0.4 | 0.8 |
| *Serratia marcescens* SANK 73060 | >200 | >200 |

## Biological Properties

As shown in Table 2, MRDs E and F were not active against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Serratia marcescens* but selectively active against *P. aeruginosa*. They showed similar antimicrobial spectra to those of MRD A but were a little weaker.

## Chemical Synthesis of MRDs E and F from MRD A

By the addition of formaldehyde to an aqueous solution of MRD A followed by Toyopearl column chromatographic separation, MRDs E and F were obtained as mentioned in the section of Materials and Methods. These compounds showed the same physico-chemical and biological properties as those from culture filtrate. Employing acetaldehyde instead of formaldehyde, methyl MRDs E and F were synthesize

but their biological activities were much less than those of MRDs E and F.

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