Thiomarinols B and C, New Antimicrobial Antibiotics Produced by a Marine Bacterium

Sir:

In our previous papers, we reported the structure and stereochemistry of thiomarinol A (previously referred to as thiomarinol)[1,2], produced by a marine bacterium, *Alteromonas rava* sp. nov. SANK 73390[3], which has antimicrobial activity against Gram-positive and Gram-negative bacteria. Thiomarinol A (TMA) is a hybrid antibiotic composed of a pseudomonic acid analogue and holothin. In this paper we report the isolation, structure elucidation and antimicrobial activities of new thiomarinol derivatives, thiomarinols B (TMB)[4] and C (TMC)[5].

TMA was isolated from EtOAc extracts of culture broth of SANK 73390[1]. TMB and TMC were separated from the residue containing minor components, obtained from the extracts by removing TMA on column chromatography with silica gel. The residue was dissolved in methanol and applied on preparative HPLC, using a reverse phase column (Senshu-Pak, ODS, H-4251, 10 × 250 mm) and developed with 40% CH_3CN at a flow rate of 5 ml/minutes. Two main peaks were collected, and TMB and TMC were obtained as yellow powders by condensation *in vacuo*, and lyophilization.

The physico-chemical properties of TMB and TMC are summarized in Table 1.

On comparison of the molecular formulae of TMA (**1**) and TMB (**2**), **2** was found to have two more oxygen atoms than **1**. The ^1H and ^{13}C NMR spectra of **2** were closely similar to those of **1**. However, the chemical shifts of the two sp^2 carbons at C-3″ and C-4″ in the holothin part of **2** were different from those of **1**. The UV absorption of **2** was also different from that of **1**. These observations suggested that an additional two oxygen atoms were shared with the chromophore part. The structure of the monic acid moiety of **2** was identical with 4-hydroxymonic acid C (**4**)[1] of **1** from NMR analyses. Additionally, the 4-hydroxymonic acid C was identified by HPLC analysis of the hydrolysis products of **1** and **2** with mild alkali (0.02 N NaOH) in 60% CH_3CN-H_2O at 10°C overnight. On the other hand, the acylchromophore moiety (**6**) of **2**, which was obtained from hydrolysis with alkali as mentioned above, was different from the 8-hydroxyoctanoylholothin (**5**) of **1**. HRFAB-MS spectra of **6** showed peaks at *m/z* 347.0737 ($C_{13}H_{19}O_5N_2S_2$) and at *m/z* 203.9664 ($C_5H_4O_3N_2S_2$), corresponding to the molecular ion ($M+H^+$) and holothin part. These ions were larger by 32 mass units than those of **5**. This fact led us to the conclusion that 8-hydroxyoctanoic acid was the same as **5** and the chromophore part (holothin) of **6** possessed an additional two oxygens. The differences in the UV spectra and chemical and other spectral properties between **1** and **2**, as mentioned above, suggested the presence of sulfoxides or a sulfone structure produced by oxidation of the disulfide part of **1**.

The complete structure of **2** was deduced by X-ray analysis. A yellow crystal with approximate dimensions 0.5 × 0.4 × 0.2 mm was obtained by recrystallization from methanol-H_2O solution. The crystal data are as follows: $C_{30}H_{44}N_2O_{11}S_2 \cdot CH_3OH$, MW = 704.85, monoclinic, $P2_1$, a = 27.088(9) Å, b = 5.937(1) Å, c = 10.925(5) Å, β = 91.41(3)°, V = 1756.6(9) Å^3, Z = 2, D_{calc} = 1.33 g·cm^{-3}. The structure was solved by MULTAN 78 and refined by block-diagonal least-squares refinement to R = 0.048 for 2705 observed reflections ($F_0 \geq 3\sigma(F_0)$). The absolute configuration of **2** was determined using anomalous scattering effects of the sulfur atoms. Methanol as the crystal solvent forms two hydrogen-bonds to the antibiotic, which stabilizes the crystal

Table 1. Physico-chemical properties of thiomarinol B and C.

		B	C
Molecular formula		$C_{30}H_{44}N_2O_{11}S_2$	$C_{30}H_{44}N_2O_8S_2$
Molecular weight		672	624
HR-FAB/MS $(M+H)^+$	Found:	673.2468	625.2634
	Calcd.:	673.2465	625.2618
Elemental analysis	Found (%):	C: 52.34	C: 56.48
		H: 6.79	H: 7.23
		N: 3.92	N: 4.30
		S: 9.02	S: 9.11
	Calcd. (%):	$C_{30}H_{44}N_2O_{11}S_2 \cdot H_2O$	$C_{30}H_{44}N_2O_8S_2 \cdot H_2O$
		C: 52.16	C: 56.05
		H: 6.71	H: 7.21
		N: 4.06	N: 4.36
		S: 9.28	S: 9.97
$[\alpha]_D^{25}$		+7.7° (1-propanol; *c* 1.0)	−1.4° (methanol; *c* 1.0)
UV λ_{max} nm (ε)		215 (21000)	215 (17000)
		301 (13000)	300 (2700)
		377 (2900)	388 (9600)
HPLC* Rt (minutes)		8.4	11.3

* Senshu-Pak, ODS H-2151 (6 × 150 mm), developing solvent: 40% CH_3CN, 1.5 ml/minute.

Fig. 1. Structures of TMA (**1**), B (**2**) and C (**3**) and their degradation produts.

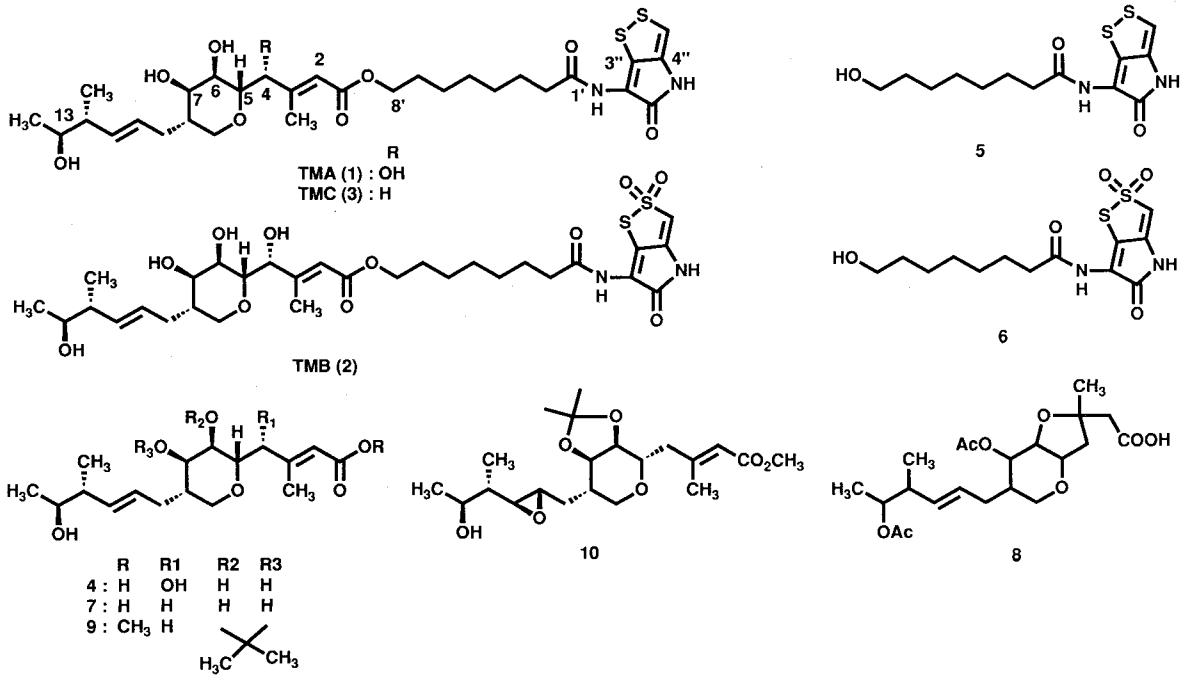
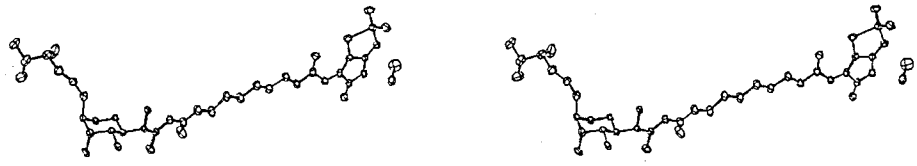


Fig. 2. Stereoscopic drawing of thiomarinol B (**2**).



structure. Fig. 2 is a stereoscopic drawing of **2**. The absolute configuration of the 4-hydroxymonic acid C in **2** was the same as that of **1**, which was elucidated by modified Mosher's method[2] except for at C-12. The configuration and geometries of **2**, except at C-4, are the same as those of pseudomonic acid C[6].

Finally, the structure advanced for **2** was supported by an unambiguous synthesis. After oxidation of **1** with oxone in 50% acetone-water, to presumably obtain an unstable sulfoxide species, a dilute solution of $NaHCO_3$ was added at ice-bath temperature and then stirred for 1 hour followed by extraction with CH_2Cl_2 and then preparative HPLC to give **2** in 75% yield[7]. The synthetic compound was found to be identical with the natural product in all spectral data in addition to biological activity.

TMC (**3**) had the same UV absorption as **1**. On comparison of the molecular formulae of **1** and **3**, **3** had one less oxygen atom than **1**. Acetylation of **3** with acetic anhydride in pyridine gave a tetraacetate [*m/z* 793 ($M+H^+$) in FAB-MS], in contrast to the pentaacetate[1] of **1**. These facts suggest that **3** is a monodeoxy derivative of **1**. On comparison of the ^1H NMR spectrum of **3** with that of **1**, it was noted that a methine signal at 4.18 ppm (4-H) in **1** was not present in **3**, and instead an AB type of methylene signal appeared at 2.56 and 2.15 ppm, coupled with the 2-H and 5-H protons. On the other hand, in the ^{13}C NMR spectrum of **3**, one of five methine carbons bearing an oxygen (C-4, C-5, C-6, C-7, and C-13) in **1** disappeared and a methylene carbon appeared at 42.5 ppm as a triplet. Mild alkali hydrolysis of **3**, as mentioned above, gave the same acylchromophore (**5**), but its monic acid moiety (**7**) was different from the 4-hydroxymonic acid C (**4**) in HPLC analysis. The molecular ion ($M+H^+$) of the new monic acid (**7**), which was obtained by preparative HPLC of hydrolysate of **3**, was at *m/z* 329 in FAB-MS. Alkali hydrolysis of **3** with 0.1 N NaOH in 70% methanol-H_2O at 50°C for 3 hours followed by acetylation gave a diacetate of bicyclic compound (**8**), which had been derived from monic acid C[6]. In the ^1H NMR spectrum of **8**, two protons at 7-H and 13-H shifted down field at 5.25 and 4.80 ppm on the acetylation, and the 4-H protons at 2.11 ppm coupled with 5-H at 3.82 ppm. These results suggest that this monic acid (**7**) is identical to monic acid C. Therefore, the structure of **3** was deduced as 4-deoxythiomarimol A as shown in Fig. 1.

The absolute configuration of monic acid C in **3** was

confirmed by the relation to the monic acid A in pseudomonic acid A[8]. Hydrolysis of 6,7-acetonide of **3** with 0.1 N NaOH in 70% MeOH solution, which was prepared by reaction with 2,2-dimethoxypropane in the presence of *p*-TsOH, afford a methyl monate C 6,7-acetonide (**9**). Epoxidation of **9** with 3-chloroperoxybenzoic acid (*m*CPBA) in CH_2Cl_2 solution gave two peaks in HPLC analysis (Senshu Pak, Aquasil, SS-352N(B), 4.6×250 mm, developing solvent; CH_2Cl_2-CH_3CN (8:2 v/v) containing H_2O (0.6%)). One of the peaks was identical with the methyl monate A 6,7-acetonide (**10**) and the other was the isomer at epoxide orientation. The authentic sample of **10** was prepared by reaction of pseudomonic acid A[8] with 2,2-dimethoxypropane in the presence of *p*-TsOH and followed hydrolysis with 0.1 N NaOH in 70% MeOH as mentioned above. We isolated **10** and its isomer by preperative HPLC using Aquasil SS-352N(B) as mentioned above condition. **10** from TMC was identical with the authentic sample of methyl monate A 6,7-acetonide with respects to spectral data including the optical rotation. The geometry of double bond at C-10~11 was assigned as *E* by ^{1}H NMR spectrum. The coupling constants between 10-H (dt) at 5.56 and 11-H (dd) at 5.77 ppm of **3** in pyridine-d_5 was observed at 15.5 Hz. This value was assigned as signifying *trans*. Therefore, the absolute configuration of monic acid C of **3** was identical with monic acid C of pseudomonic acid C.

Thiomarinol B showed excellent *in vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria (Table 2). Among these bacteria, it was especially active against *S. aureus* including MRSA similar to TMA. Thiomarinol C was slightly less active than TMA.

Table 2. Antimicrobial Activities of Thiomarinol B (**2**) and C (**3**).

Test organism	MIC (μg/ml)	
	2	**3**
Staphylococcus aureus 209P JC-1	≤0.01	≤0.01
S. aureus 56R	≤0.01	≤0.01
S. aureus 535 (MRSA)	≤0.01	≤0.01
Enterococcus faecalis 681	0.05	0.8
Escherichia coli NIHJ JC-2	0.8	3.1
E. coli 609	0.8	1.5
Salmonella enteritidis	0.4	1.5
Klebsiella pneumoniae 806	0.8	1.5
K. pneumoniae 846 (R)	0.2	0.8
Enterobacter cloacae 963	0.8	3.1
Serratia marcescens IAM1184	3.1	6.2
Proteus vulgaris 1420	0.05	0.2
Morganella morganii 1510	6.2	12.5
Pseudomonas aeruginosa 1001	0.2	0.8
P. aeruginosa No. 7	0.4	0.8
P. aeruginosa PA01	0.8	0.4

The minimum inhibitory concentrations (MIC) were determined by a serial 2-fold plate dilution method with a nutrient agar (Eiken Chemical Co., Ltd.) on which 1 loopful 10^7 cfu/ml suspension of test bacteria was streaked, followed by incubation at 37°C for 20 hours.

HIDEYUKI SHIOZAWA
TAKESHI KAGASAKI
AKIO TORIKATA
NATSUKI TANAKA†
KATSUMI FUJIMOTO†
TADASHI HATA††
YOUJI FURUKAWA††
SHUJI TAKAHASHI*

Biomedical Research Laboratories,
†Exploratory Chemistry Research Laboratories,
††Analytical and Metabolic Research Laboratories,
Sankyo Co., Ltd.,
2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan

(Received March 6, 1995)

References

1) SHIOZAWA, H.; T. KAGASAKI, T. KINOSHITA, H. HARUYAMA, H. DOMON, Y. UTSUI, K. KODAMA & S. TAKAHASHI: Thiomarinol, a new hybrid antimicrobial antibiotic produced by a marine bacterium. J. Antibiotics 46: 1834~1842 1993
2) SHIOZAWA, H. & S. TAKAHASHI: Configurational studies on thiomarinol. J. Antibiotics 47: 851~853, 1994
3) KODAMA, K.; H. SHIOZAWA & A. ISHII: *Alteromonas rava* sp. nov., a marine bacterium that produces a new antibiotic, thiomarinol. Annu. Rep. Sankyo Res. Lab. 45: 131~136, 1993
4) TAKAHASHI, S.; H. SHIOZAWA, T. KAGASAKI, K. OGAWA, K. KODAMA & A. ISHII (Sankyo Co., Ltd.): New antibiotic, thiomarinol B and their preparation. Jpn. Kokai. 336,487, Dec. 6, 1994
5) TAKAHASHI, S.; H. SHIOZAWA, K. OGAWA, Y. SAKAIDA, A. TORIKATA, K. KODAMA & A. ISHII (Sankyo Co., Ltd.): New antibiotic, Thiomarinol C and their preparation. Jpn. Kokai. 199,865, July 19, 1994
6) CLAYTON, J. P.; P. J. O'HANLON, N. H. ROGERS & T. J. KING: The chemistry of pseudomonic acid. Part 5. Structure and chemistry of pseudomonic acid C. X-ray crystal structure of ethyl monate C. J. Chem. Soc. Perkin Trans. I: 2827~2833, 1982
7) FUJIMOTO, K.; Y. IWANO & K. HIRAI (Sankyo Co., Ltd.): Preparation of thiomarinol B. Jpn. Kokai. 206,884, July 26, 1994
8) ALEXANDER, R. J.; J. P. CLAYTON, K. LUK, N. H. ROGERS & T. J. KING: The chemistry of pseudomonic acid, Part 1. The absolute configuration of pseudomonic acid A. J. Chem. Soc. Perkin Trans. I: 561~565, 1978