COMMUNICATIONS TO THE EDITOR

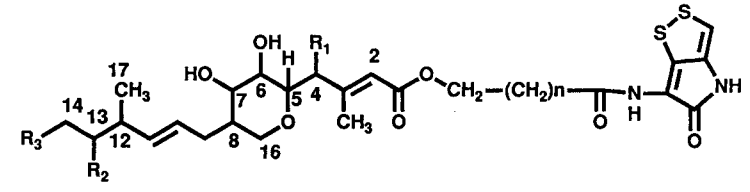
Thiomarinols D, E, F and G, New Hybrid Antimicrobial Antibiotics Produced by a Marine Bacterium; Isolation, Structure, and Antimicrobial Activity

Sir:

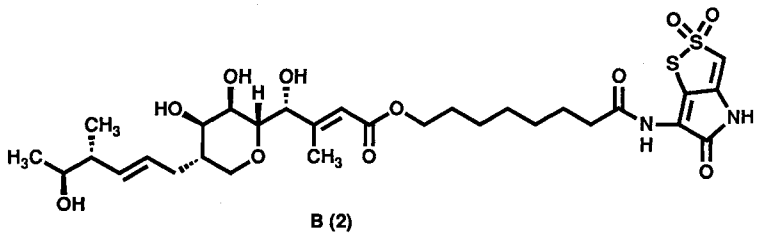
In our previous papers, we reported new antibiotics, thiomarinol A (TMA, **1**)[1] and minor components thiomarinols B (TMB, **2**) and C (TMC, **3**)[2], which were isolated from the culture broth of a marine bacterium, *Alteromonas rava* sp. nov. SANK 73390. Thiomarinols A and C are hybrid antibiotics composed of a pseudomonic acid analogue and holothin[3]. In this paper, we report isolation, structure elucidation and antimicrobial activities of new minor components, thiomarinols D (TMD), E (TME), F (TMF) and G (TMG).

Isolation procedures for TMD (**4**), TME (**5**), TMF (**6**) and TMG (**7**) were similar to those for TMB and TMC[2]. TMA was a major product and isolated from EtOAc extracts of the culture broth[1]. The residue obtained from the extracts by removing TMA on column chromatography with silica gel contained the minor components, and was used for the source of them. The residue (25 g) was dissolved in MeOH and applied on a cosmosil column (140C18-OPN, nacalai tesque). After washing with 35% CH_3CN, minor components, **4**, **5**, **6** and **7** were eluted with 40% CH_3CN, followed by concentration *in vacuo* and lyophilization, respectively. The each fraction was purified with a Sephadex LH-20 column developed with CH_2Cl_2-EtOAc-MeOH (200:200:30 v/v), and finally applied on preparative HPLC using a reverse phase column (Senshu-pak, ODS, H-4251,

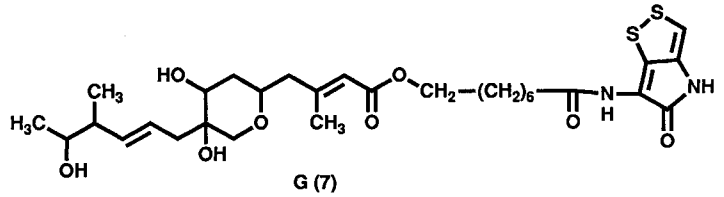
Fig. 1. Structures of thiomarinols and acylchromophores.



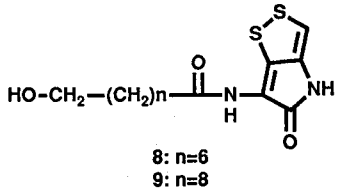
	n	R1	R2	R3
A (1):	6	OH	OH	H
C (3):	6	H	OH	H
D (4):	6	OH	OH	CH3
E (5):	8	OH	OH	H
F (6):	6	OH	=O	H



B (2)



G (7)



8: n=6
9: n=8

Table 1. Physico-chemical properties of thiomarinols D (**4**), E (**5**), F (**6**) and G (**7**).

	4	**5**	**6**	**7**
Molecular formula:	$C_{31}H_{46}N_2O_9S_2$	$C_{32}H_{48}N_2O_9S_2$	$C_{30}H_{42}N_2O_9S_2$	$C_{30}H_{44}N_2O_8S_2$
M.W:	654	668	638	624
HR FAB-MS ($M+H^+$):	$C_{31}H_{47}N_2O_9S_2$	$C_{32}H_{49}N_2O_9S_2$	$C_{30}H_{43}N_2O_9S_2$	$C_{30}H_{45}N_2O_8S_2$
Found	655.2723	669.2875	639.2411	625.2601
Calcd.	655.2723	669.2880	639.2410	625.2618
$[\alpha]_D^{25°}$ (MeOH);	+1.48° (*c*, 0.81)		−1.66° (*c*, 0.78)	
UV; λ (MeOH), nm (ε):	210 (20900)	210	210 (21200)	210
	300 (2600)	300	300 (2600)	300
	385 (8900)	385	385 (8500)	385
IR; ν cm^{-1} (KBr)	3401, 1651		3408, 1698	
	1598, 1526		1652, 1598	
	1457, 1290		1523, 1455	
	1216, 1155		1290, 1216	
HPLC (Rt, minutes)[a]	8.7	11.8	6.7	11.2

[a] YMC-pak A-312, 6 × 150 mm, developing solvent: 40% CH_3CN, 1.5 ml/minute.

10 × 250 mm) developed with 40% CH_3CN. **4**, **5**, **6**, and **7** were obtained as 16 mg, 37 mg, 27 mg and 26 mg of yellow powder by lyophilization, respectively.

Physico-chemical properties, ^1H and ^{13}C NMR data of TMD, TME, TMF and TMG are summarized in Tables 1, 2 and 3.

The UV spectra with maxima at 210, 300 and 385 nm of **4**, **5**, **6** and **7** were identical with that of **1**. Mild alkali hydrolysis of **4**, **6** and **7** gave an acylchromophore (**8**), which was identical with that obtained by the hydrolysis of **1**[1]. These results suggested that the right wing of structure, acylchromophore moieties of **4**, **6**, and **7** were the same as that of **1**. Therefore, the left wing of structure, the monic acid moieties of **1**, **4**, **6**, and **7** must be different from each other. On the other hand, mild alkali hydrolysis of **5** gave a new acylchromophore (**9**) [*m/z*: 343 ($M+H^+$) in FAB-MS], which was deduced as 10-hydroxydecanoylholothin by MS and NMR spectra. The monic acid moiety of **5** was identical with that of **1**. Therefore, the structure of **5** was deduced as a thiomarinol A derivative having two more methylenes than thiomarinol A in the acylchromophore part.

Two doublet signals of methyl groups at C-14 and C-17 in the ^1H NMR in **1** were changed to one doublet and one triplet methyl signals in that of **4**. Acetylation of **4** with acetic anhydride in pyridine gave a pentaacetate (**10**). In the DQF-COSY of **10**, a methine proton at 4.73 ppm (13-H), shifted to lower field on the acetylation, was coupled with a methine proton at 2.37 ppm (12-H), which was further coupled with a methyl signal at 0.98 ppm (17-CH_3). The methine proton (13-H) was also coupled with a methylene signal at 1.51 ppm (14-H), which was further coupled with methyl signal at 0.85 ppm (18-CH_3) as shown in Fig. 2a. The linkages from 2-H* to 12-H of the monic acid part in **10** directly established the same partial structure of **1** as shown in Fig. 2b (*: A long range coupling between 2-H and 4-H as well as

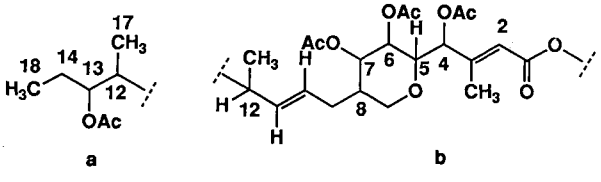
Table 2. ^{13}C NMR data of thiomarinols D (**4**), E (**5**), F (**6**) and G (**7**).

4 (DMSO-d_6)	**5** (CD_3OD)	**6** (CD_3OD)	**7** (CD_3OD)
171.7 (s)	174.4 (s)	212.4 (s)	174.8 (s)
167.8 (s)	170.4 (s)	174.3 (s)	170.5 (s)
166.0 (s)	168.6 (s)	170.4 (s)	168.3 (s)
160.8 (s)	161.2 (s)	168.6 (s)	158.2 (s)
133.9 (d)	138.9 (s)	161.1 (s)	138.0 (s)
133.8 (s)	135.7 (d)	137.9 (s)	137.4 (d)
133.6 (s)	135.1 (s)	135.1 (s)	135.2 (s)
127.6 (d)	129.8 (d)	132.5 (d)	126.2 (d)
115.2 (s)	116.2 (d)	132.4 (d)	118.6 (d)
114.2 (d)	115.8 (s)	116.2 (d)	115.9 (s)
110.4 (d)	113.6 (d)	115.8 (s)	113.6 (d)
76.1 (d)	77.5 (d)	113.6 (d)	72.2 (d)
74.9 (d)	74.4 (d)	77.5 (d)	71.2 (s)
72.3 (d)	72.1 (d)	74.3 (d)	71.1 (d)
69.5 (d)	71.8 (d)	71.7 (d)	70.4 (d)
64.1 (t)	65.9 (t)	65.8 (t)	70.0 (t)
63.8 (d)	65.7 (d)	65.6 (d)	64.8 (t)
62.9 (t)	64.9 (t)	64.8 (t)	47.3 (t)
42.1 (d)	45.3 (d)	52.0 (d)	45.5 (d)
41.7 (d)	44.9 (d)	43.7 (d)	39.8 (t)
34.5 (t)	36.6 (t)	36.5 (t)	37.0 (t)
31.8 (t)	33.4 (t)	33.2 (t)	36.6 (t)
28.3 (t)	30.4 (t)	30.0 (t)	30.1 (t)
28.2 (t)	30.2 (t)x2	29.9 (t)	30.0 (t)
28.1 (t)	30.1 (t)	29.7 (t)	29.8 (t)
26.8 (t)	29.8 (t)	28.1 (q)	26.9 (t)
25.2 (t)	27.1 (t)	26.9 (t)	26.7 (t)
24.8 (t)	26.7 (t)	26.6 (t)	20.3 (q)
16.9 (q)	20.3 (q)	16.4 (q)	19.3 (q)
15.6 (q)	16.6 (q)	16.2 (q)	16.6 (q)
10.4 (q)	16.3 (q)		

Table 3. ^{1}H NMR data of thiomarinols D (**4**), E (**5**), F (**6**) and G (**7**) (CH_3OD, 400 MHz).

4	5	6	7
7.08 (1H, s)	7.08 (1H, s)	7.08 (1H, s)	7.06 (1H, s)
6.08 (1H, s)	6.07 (1H, s)	6.08 (1H, s)	5.71 (1H, s)
5.45 (2H, m)	5.46 (2H, m)	5.63 (1H, m)	5.54 (1H, dt, J=15.6, 6.6)
4.33 (1H, s)	4.35 (1H, s)	5.52 (1H, m)	5.48 (1H, dt, J=15.6, 7.2)
4.09 (2H, t, J=6.4)	4.08 (2H, t, J=6.4)	4.35 (1H, s)	4.07 (2H, t, J=6.4)
3.92 (1H, b-s)	3.92 (1H, b-s)	4.10 (2H, m)	3.92 (1H, m)
3.84 (1H, dd, J=9.6, 3.2)	3.84 (1H, dd, J=9.5, 3.2)	3.91 (1H, b-s)	3.73 (1H, t, J=0.5)
3.77 (1H, dd, J=11.3, 2.5)	3.78 (1H, dd, J=11.4, 2.5)	3.85 (1H, dd, J=9.5, 3.0)	3.61 (1H, m)
3.70 (1H, dd, J=9.6, 1.7)	3.70 (1H, dd, J=9.5, 1.5)	3.79 (1H, dd, J=11.5, 2.3)	3.50 (1H, d, J=11.2)
3.54 (1H, d, J=11.3)	3.60 (1H, m)	3.70 (1H, dd, J=9.5, 1.5)	3.39 (1H, d, J=11.2)
3.29 (1H, m)	3.54 (1H, d, J=11.4)	3.50 (1H, d, J=11.5)	2.38 (2H, t, J=7.4)
2.40 (2H, t, J=7.4)	2.40 (2H, t, J=7.4)	3.25 (1H, m)	2.35 (4H, m)
2.25 (1H, m)	2.25 (1H, m)	2.40 (2H, t, J=7.4)	2.26 (1H, dd, J=14.0, 4.4)
2.15 (2H, m)	2.17 (2H, m)	2.28 (1H, m)	2.15(3H, s)
2.13 (3H, s)	2.14 (3H, s)	2.18 (1H, m)	1.66(6H, m)
1.70 (5H, m)	1.65 (5H, m)	2.12 (3H, s) ×2	1.39(6H, m)
1.40 (8H, b-s)	1.35 (10H, b-s)	1.65 (5H, m)	1.10 (3H, d, J=6.4)
1.01 (3H, d, J=6.9)	1.09 (3H, d, J=6.4)	1.40 (6H, b-s)	1.00 (3H, d, J=7.2)
0.94 (3H, t, J=7.3)	0.98 (3H, d, J=6.7)	1.11 (3H, d, J=6.8)	

Fig. 2. Partial structures of thiomarinol D pentaacetate (**10**).



vicinal couplings was observed). Therefore, the structure of **4** was deduced as 14-homothiomarinol A as shown in Fig. 1.

On comparison of the molecular formulae, **6** was found to have two less protons than **1**. Acetylation of **6** with acetic anhydride in pyridine gave a tetraacetate (**11**) [*m/z*: 807 ($M+H^+$) in FAB-MS], in contrast to the pentaacetate of **1**. In the ^{13}C NMR spectrum of **6**, a ketone carbonyl appeared at 212.4 ppm, and one of five methine carbons having an oxygen (C-4, C-5, C-6, C-7 and C-13) disappeared compared with that of **1**. One of two doublet methyl signals of **1** changed to a singlet methyl signal at 2.12 ppm in the ^{1}H NMR spectrum. These facts suggest that **6** is an oxydation compound of the hydroxy group at C-13 in **1**. **6** had the same acylchromophore (**8**) in right wing of the structure as mentioned above. Therefore, the structure of **6** was deduced as 13-ketothiomarinol A as shown in Fig. 1.

TMG (**7**) and TMC (**3**) had the same molecular formula and the same acylchromophore (**8**). On comparison of ^{13}C NMR spectra of **7** and **3**, **7** had one more quaternary carbon at 71.2 ppm, bearing an oxygen atom and lost one of four methine carbons (C-5, C-6, C-7 and C-13). Acetylation of **7** with acetic anhydride in pyridine gave a triacetate (**12**) [*m/z*: 751 ($M+H^+$) in FAB-MS], in contrast to the tetraacetate of **3**. The structure of the monic acid part of **7** was different from that of **3**. Partial structures 3a, 3b and 3c of **12** as shown in Fig. 3 were derived from the ^{1}H-^{1}H connectivities by DQF-COSY spectra combined with the ^{1}H-^{13}C HMQC spectra. The linkages of the partial structures 3a, 3b and 3c were established from HMBC spectra. Cross peaks from 16-H_2 to C-5, C-7, C-8 and C-9, from 7-H to C-8, and from 9-H to C-8 were observed as shown as arrow lines in Fig. 3. From the above spectral data and biogenetically consideration, the structure of monic acid part of **7** was assumed to be 6-deoxy-8-hydroxymonic acid C as shown in Fig. 1. An 8-hydroxymonic acid derivative was already reported as pseudomonic acid B[4].

Therefore, the structures of TMD, E, F, and G were described as **4**, **5**, **6**, and **7** as shown in Fig. 1.

TMD, E, F and G exhibited antimicrobial activities against Gram-positive and Gram-negative bacteria as shown in Table 4. They were especially active against Gram-positive bacteria including MRSA. Among them, TME showed the most potent activity. It was as active as TMA. TMG showed the least potent activity. The mode of action of pseudomonic acid A is inhibition of isoleucyl-transfer RNA synthetase in bacteria[5]. Thiomarinols also inhibited the same enzyme strongly. TMD, which had a homoisoleucine type structure, as well as TMA, TMB, and psuedomonic acid A, specifically

Fig. 3. Partial structures of thiomarinol G triacetate (**12**).

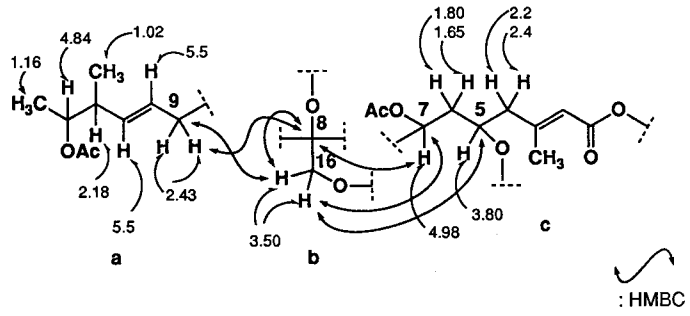


Table 4. Antimicrobial activities of thiomarinols D (**4**), E (**5**), F (**6**) and G (**7**).

Test organism	MIC (μg/ml)			
	4	**5**	**6**	**7**
Staphylococcus aureus 209P JC-1	≤0.01	≤0.01	≤0.01	≤0.01
S. aureus 56R	≤0.01	≤0.01	≤0.01	≤0.01
S. aureus 535 (MRSA)	≤0.01	≤0.01	≤0.01	≤0.01
Enterococcus faecalis 681	0.2	0.05	0.2	3.1
Escherichia coli NIHJ JC-2	1.5	1.5	6.2	>200
E. coli 609	1.5	0.8	3.1	50
Salmonella enteritidis	0.8	0.8	3.1	25
Klebsiella pneumoniae 806	0.8	0.8	3.1	12.5
K. pneumoniae 846 (R)	0.4	0.4	1.5	6.2
Enterobacter cloacae 963	1.5	1.5	12.5	>200
Serratia marcescens IAM1184	6.2	6.2	25	>200
Proteus vulgaris 1420	0.1	0.2	1.5	3.1
Morganella morganii 1510	12.5	12.5	100	>200
Pseudomonas aeruginosa 1001	0.4	0.4	3.1	12.5
P. aeruginosa No. 7	1.5	0.4	3.1	12.5
P. aeruginosa PA01	0.4	0.4	6.2	12.5

inhibited bacterial isoleucyl-tRNA synthetase, whereas they did not inhibit leucyl, valyl, and phenylalnyl-tRNA synthetases. These inhibitory activities of thiomarinols against the enzymes will be reported in detail elsewhere.

HIDEYUKI SHIOZAWA
ATSUKO SHIMADA
SHUJI TAKAHASHI*

Biomedical Research Laboratories,
Sankyo Co., Ltd.,
2-58 Hiromachi, 1-chome, Shinagawa-ku,
Tokyo 140, Japan

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References

1) SHIOZAWA, H.; T. KAGASAKI, T. KINOSHITA, H. HARUYAMA, H. DOMON, Y. UTUI, K. KODAMA & S. TAKAHASHI: Thiomarinol, a new hybrid antimicrobial antibiotic produced by a marine bacterium. Fermentation, isolation, structure and antimicrobial actibity. J. Antibiotics 46: 1834~1842, 1993
2) SHIOZAWA, H.; T. KAGASAKI, A. TORIKATA, N. TANAKA, K. FUJIMOTO, T. HATA, Y. FURUKAWA & S. TAKAHASHI: Thiomarinols B and C, new antimicrobial antibiotics produced by a marine bacterium. J. Antibiotics 48: 907~909, 1995
3) ETTLINGER, L.; E. GÜUMANN, R. HÜTTER, W. KELLER-SCHIERLEIN, F. KRADOLFER, L. NEIPP, V. PRELOG & H. ZÄHNER: Stoffwechselprodukte von Actinomyceten, Holomycin. Helv. Chim. Acta 42: 563~569, 1959
4) CHAIN, E. B. & G. MELLOWS: Pseudomonic acid. Part 3. Structure of pseudomonic acid B. J. Chem. Soc. Perkin Trans. I 1977: 318~322, 1977
5) HUGHES, J. & G. MELLOWS: Interaction of pseudomonic acid A with *Escherichia coli* B isoleucyl-tRNA synthetase. Biochem. J. 191: 209~219, 1980