Structures of Novel Epipolythiodioxopiperazines, Emethallicins B, C, and D, Potent Inhibitors of Histamine Release, from *Emericella heterothallica*[1)]

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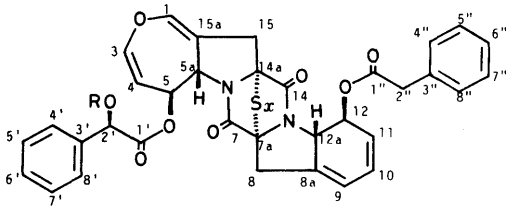
Novel compounds designated emethallicins B (1), C (2), and D (3), along with emethallicin A (4), were isolated from the mycelium of the heterothallic fungus, *Emericella heterothallica* (mating type a). The structures of emethallicins B (1), C (2), and D (3) were determined on the basis of spectroscopic and chemical investigations. Emethallicins B (1) and C (2) are epitetrathiodioxopiperazines, which have the same basic carbon skeleton as apoaranotin (19) and acetylaranotin (17), respectively, whereas emethallicin D (3) is an epitrithiodioxopiperazine derivative, which has the same carbon skeleton as apoaranotin (19). It is very interesting that a large amount of the disulfide, emethallicin A (4), was isolated from the strain of mating type A and that the corresponding tetrasulfide, emethallicin B (1), and trisulfide, emethallicin D (3), were isolated from the other mating type strain, along with a small amount of the disulfide (4). Emethallicins B (1), C (2), and D (3) have potent inhibitory activity against compound 48/80-induced histamine release from mast cells, like emethallicin A (4).

Keywords *Emericella heterothallica*; heterothallic fungus; epitetrathiodioxopiperazine; epitrithiodioxopiperazine; emethallicin A; emethallicin B; emethallicin C; emethallicin D; histamine release inhibition

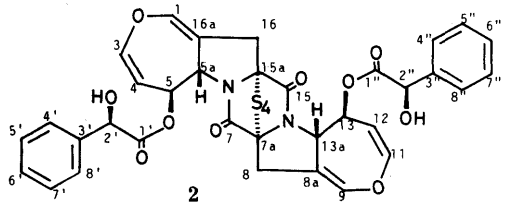
The isolation of an epidithiodioxopiperazine derivative designated emethallicin A (**4**) from the mycelial chloroform extract of *Emericella* (*E.*) *heterothallica* (KWON, FENNELL & RAPER) MALLOCH & CAIN (anamorph: *Aspergillus heterothallicus* KWON, FENNELL & RAPER), strain ATCC 16847 (mating type A), as the main component, was previously reported.[2)] In the course of a search for other epipolythiodioxopiperazine derivatives, two new epitetrathiodioxopiperazines, emethallicins B (**1**) and C (**2**), were isolated from the mycelial chloroform extract of *E. heterothallica*, strain ATCC 16824 (mating type a), along with a small amount of emethallicin A (**4**).[3)] In addition, a new epitrithiodioxopiperazine designated emethallicin D (**3**) was isolated as its monoacetate (**5**) upon purification after acetylation of the **4**-rich fraction from the above extract. The structures of emethallicins B (**1**), C (**2**), and D (**3**) are reported in this paper.

Emethallicins B (**1**), $[\alpha]_D$ $-268°$ ($CHCl_3$), and C (**2**), $[\alpha]_D$ $-312°$ ($CHCl_3$), gave quasi-molecular ions at *m/z* 721 $(M+1)^+$ and 753 $(M+1)^+$, respectively, by fast-atom bombardment (FAB) mass spectrometry, and elemental analyses confirmed their molecular formulae as $C_{34}H_{28}N_2O_8S_4$ and $C_{34}H_{28}N_2O_{10}S_4$, respectively. The molecular formula of emethallicin D monoacetate (**5**), $[\alpha]_D$ $-269°$ ($CHCl_3$), was also confirmed as $C_{36}H_{30}N_2O_9S_3$ by FAB mass spectrometry [731 $(M+1)^+$] and elemental analysis. A positive coloration with silver nitrate (dark brown-black)[2,4)] suggested the presence of the tetrasulfide bond in **1** and **2** and the trisulfide bond in **5**. The infrared (IR) absorption maxima of **1** (1735 and 1720, and 1680 cm^{-1}), **2** (1730 and 1680 cm^{-1}), and **5** (1740 and 1700 cm^{-1}) suggested the presence of both esters and amides in each compound. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals at δ 165.29 and 166.16 for **1**, at δ 165.38 (2C) for **2**, and δ 163.55 (162.93) and 163.95 (164.81) for **5** (Tables I and II) were assigned to two amide carbonyl carbons in each compound, in view of the presence of two nitrogen atoms in the molecule of **1**, **2**, and **5**, whereas the ^{13}C-NMR signals at δ 170.33 and 171.55 for **1**, at δ 171.66 (2C) for **2**, and at δ 171.03 (170.90) and 167.62 (168.03) for **5** (Tables I and II) were assigned to two ester carbonyl carbons. The ^{13}C-NMR signal at δ 169.81 (170.14) (qd) for **5** was assigned to the ester carbonyl carbon of the acetate from the coupling pattern. The above results suggested that emethallicins B (**1**) and C (**2**), and emethallicin D monoacetate (**5**) had epitetrathiodioxopiperazine moieties and an epitrithiodioxopiperazine moiety, respectively, in the molecule.

All of the proton nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR signals of emethallicin B monoacetate (**1**) were similar to those of emethallicin A (**4**) (Tables I and III). On acetylation, **1** afforded a monoacetate (**6**), $[\alpha]_D$



1 : R=H, x=4 **5** : R=Ac, x=3
3 : R=H, x=3 **6** : R=Ac, x=4
4 : R=H, x=2 **7** : R=Ac, x=2



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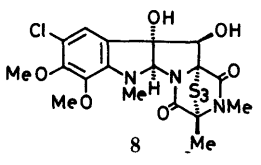

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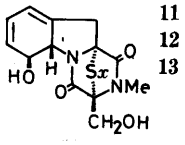

11 : x=3
12 : x=2
13 : x=4

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of Emethallicins and Their Acetates in $CDCl_3$

Carbon No.	1[a]	4	5		6	7
			Major	Minor		
1	137.97 (Dm[b])	139.39 (Dm)	138.28	138.89 (Dm)	138.67 (Dm)	139.20 (Dm)
3	140.24 (Dm)	141.45 (Dm)	140.39	140.48 (Dm)	139.96 (Dm)	141.22 (Dm)
4	104.66 (Ddd)	104.20 (Dm)	104.87	104.99 (Dm)	105.20 (Dm)	104.22 (Dm)
5	70.71 (Ddd)	71.55 (Dm)	71.85	72.90 (Dm)	72.14 (Dm)	70.85 (Dm)
5a	60.22 (Dm)	62.74 (Dm)	60.39	61.90 (Dm)	60.46 (Dm)	62.35 (Dm)
7	165.29 (d)	163.45 (d)	163.95	164.81 (d)	166.67 (d)	162.94 (d)
7a	80.04 (dd)	78.34 (dd)	78.58	82.33 (dd)	79.42 (dd)	78.32 (dd)
8	40.05 (Tdd)	36.08 (Td)	40.98	41.33 (Td)	41.78 (Tdd)	35.82 (Td)
8a	133.50 (m)	132.07 (m)	131.45	132.21 (m)	132.32 (m)	132.62 (m)
9	119.79 (Dm)	119.99 (Dm)	120.70	120.25 (Dm)	120.98 (Dm)	119.81 (Dm)
10	125.38 (Dm)	124.45 (Ddd)	124.40	124.50 (Dm)	124.96 (Dm)	124.53 (Ddd)
11	127.68 (Ddd)	127.64 (Dm)	127.68	127.68 (Dm)	128.95 (Dm)	127.49 (Dm)
12	74.26 (Ddd)	74.37 (Ddd)	75.48	75.32 (Dm)	74.95 (Ddd)	74.51 (Ddd)
12a	63.74 (Dm)	64.40 (Dm)	64.87	63.35 (Dm)	64.18 (Dm)	64.37 (Dm)
14	166.16 (dd)	162.06 (d)	163.55	162.93 (d)	165.76 (d)	162.32 (d)
14a	75.97 (dd)	75.73 (dd)	78.20	79.18 (dd)	75.45 (dd)	75.60 (dd)
15	40.49 (Tdd)	34.53 (Tdd)	39.63	39.55 (Tdd)	41.31 (Tdd)	34.77 (Tdd)
15a	109.50 (m)	112.73 (m)	109.26	110.03 (m)	108.49 (m)	112.90 (m)
1′	171.55 (dd)	171.70 (dd)	167.62	168.03 (dd)	168.19 (d)	167.59 (dd)
2′	72.44 (Dt)	72.86 (Dt)	73.83	73.95 (Dt)	74.28 (Dt)	73.37 (Dt)
3′	139.19 (m)	138.10 (m)	134.18	134.26 (m)	133.89 (m)[c]	134.16 (m)
4′(8′)	126.73 (Dm)	126.54 (Dm)	127.82	128.05 (Dm)	127.75 (Dm)	127.80 (Dddd)
5′(7′)	128.09 (Dd)[c]	128.35 (Dd)[c]	128.64	128.70 (Dd)[c]	128.77 (Dd)[d]	128.64 (Dd)[c]
6′	128.18 (Dm)	128.20 (Dt)	128.96	128.96 (Dt)	129.03 (Dt)	128.93 (Dt)
1″	170.33 (td)	170.90 (td)	171.03	170.90 (td)	171.00 (td)	170.93 (td)
2″	40.37 (Tt)	41.28 (Tt)	41.44	41.74 (Tt)	41.44 (Tt)	41.31 (Tt)
3″	134.18 (m)	134.09 (m)	134.18	134.26 (m)	134.08 (m)[c]	134.16 (m)
4″(8″)	129.47 (Dm)	129.52 (Dm)	129.55	129.63 (Dm)	129.68 (Dm)	129.55 (Dm)
5″(7″)	128.14 (Dd)[c]	128.40 (Dd)[c]	128.44	128.35 (Dd)[c]	128.56 (Dd)[d]	128.40 (Dd)[c]
6″	126.65 (Dt)	126.92 (Dm)	126.94	126.94 (Dm)	127.09 (Dt)	126.89 (Dm)
MeCOO			20.94	20.76 (Q)	20.91 (Q)	20.90 (Q)
MeCOO			169.81	170.14 (qd)	170.01 (qd)	169.61 (qd)

a) The spectrum of **1** was measured in $(CD_3)_2SO$. *b*) The multiplicity of the signals was determined from the proton coupled ^{13}C-NMR spectra. *c*, *d*) Assignments may be reversed.

TABLE II. ^{13}C-NMR Chemical Shifts of Emethallicin C (**2**) and Its Derivative (**18**)

Carbon No.	2[a]	18[b]
1 (9)	138.01 (Dm[c])	139.03 (Dm)
3 (11)	140.33 (Dm)	140.53 (Dm)
4 (12)	104.71 (Dm)	104.68 (Dddd)
5 (13)	70.78 (Dm)	72.46 (Dm)
5a (13a)	60.36 (Dm)	60.78 (Dm)
7 (15)	165.38 (dd)	165.43 (dd)
7a (15a)	76.22 (dd)	75.72 (dd)
8 (16)	40.41 (Tdd)	41.54 (Tdd)
8a (16a)	109.58 (m)	109.58 (m)
1′ (1″)	171.66 (dd)	163.01 (d)
2′ (2″)	72.51 (Dt)	185.24 (t)
3′ (3″)	139.24 (t)	132.50 (t)
4′,8′ (4″,8″)	126.81 (Dm)	130.38 (Dt)
5′,7′ (5″,7″)	128.18 (Dd)	128.95 (Dd)
6′ (6″)	127.80 (Dt)	134.96 (Dt)

a) The spectrum of **2** was measured in $(CD_3)_2SO$. *b*) The spectrum of **18** was measured in $CDCl_3$. *c*) The multiplicity of the signals was determined from the proton coupled ^{13}C-NMR spectra.

$-363°$ ($CHCl_3$), $C_{36}H_{30}N_2O_9S_4$. The ^{1}H-NMR and ^{13}C-NMR signals of emethallicin D monoacetate (**5**) and **6** also corresponded well to those of emethallicin A monoacetate (**7**), but most of the signals for **5** appeared as two split signals with the intensity ratio of about 2:1 when the spectrum was measured in $CDCl_3$ at room temperature (Tables I and III). This phenomenon suggested the existence of two conformers at the trisulfide part of the molecule of **5**, as reported in the case of sporidesmin E (**8**),[5] and chaetocins B (**9**) and C (**10**).[6] Determination of the ^{1}H-NMR spectrum of **5** in $C_6D_5CD_3$ at -50, 25, and 70 °C showed equilibration of the two conformers in the solution and a decrease of the minor conformer at the higher temperature (the intensity ratio between the major and minor conformers: *ca.* 3:2 at -50 °C, *ca.* 2:1 at 25 °C, and 1:0 at 70 °C). The above results suggested that emethallicin B monoacetate (**6**) and emethallicin D monoacetate (**5**) are the tetrasulfide derivative and the trisulfide derivative, respectively, of **7**.

Emethallicin D monoacetate (**5**) was treated under various basic hydrolytic conditions in order to get its naturally occurring form, *i.e.*, emethallicin D (**3**), but **3** could not be obtained. On treatment at room temperature with sodium carbonate in the mixed solution of acetone and methanol, the trisulfide (**5**) was fortunately converted to its corresponding disulfide [emethallicin A monoacetate (**7**)] along with the tetrasulfide [emethallicin B monoacetate (**6**)]. The similar disproportionation of the sulfur atoms was reported to take place when the ethanolic solution of gliotoxin E (**11**) was refluxed: the trisulfide **11** was converted to a 1:1 mixture of the disulfide, gliotoxin (**12**), and the tetrasulfide, gliotoxin G (**13**).[7] The above compounds **6** and **7** obtained from **5** by the basic treatment were identical,

TABLE III. ^{1}H-NMR Chemical Shifts of the Basic Skeleton of Emethallicins and Related Compounds in $CDCl_3$

Proton	1[a]	4	5		6	7	Proton	2[a]	17
			Major	Minor					
1-H	6.779	6.627	6.520	6.520	6.531	6.600	1-H	6.782	6.63
3-H	6.337	6.252	6.178	6.160	6.153	6.185	3-H	6.348	6.32
4-H	4.241	4.417	4.314	4.266	4.365	4.203	4-H	4.237	4.63
5-H	5.035	5.680	5.769	5.323	5.227	5.727	5-H	5.009	5.68
5a-H	5.083	5.122	5.014	5.129	5.227	5.061	5a-H	5.079	5.09
8α-H	3.075	2.843	2.893[b]	2.880[b]	3.040	3.026	8α-H	3.144	2.70
8β-H	3.451	3.772	3.414[c]	3.387[c]	3.243	3.809	8β-H	3.518	4.12
9-H	6.031	5.942[b]	5.933	5.933	5.954	5.951[b]	9-H	6.782	6.63
10-H	5.609	5.479	5.523	5.523	5.609	5.479	11-H	6.348	6.32
11-H	6.031	5.982[b]	5.933	5.933	5.929	5.987[b]	12-H	4.237	4.63
12-H	5.641	6.014	6.224	6.178	5.819	6.031	13-H	5.009	5.68
12a-H	5.261	5.020	5.349	5.349	5.422	5.027	13a-H	5.079	5.09
15α-H	3.101	2.970	2.907[b]	2.982[b]	3.067	2.966	16α-H	3.144	2.70
15β-H	3.470	4.028	3.591[c]	3.598[c]	3.289	3.990	16β-H	3.518	4.12

a) The spectra of **1** and **2** were measured in $(CD_3)_2SO$. *b*, *c*) The assignments may be reversed.

TABLE IV. The CD Maxima of Emethallicins and Related Compounds in Methanol

Compound	$[\theta]\times10^{-4}$ (nm)
Disulfides	
4[a,b]	−14.4 (219), +3.0 (268), −0.2 (338)
7[a,b]	−15.6 (218), +3.0 (267), −0.1 (337)
15[c]	−9.5 (233), +6.2 (266), +9.3 (301), −0.4 (338)
Trisulfides	
5	−8.8 (220), −8.4 (244), −0.7 (298)
16[d]	−5.7 (228), +6.9 (257), +2.0 (293)
Tetrasulfides	
1	−14.8 (226), −13.5 (236), +1.5 (272), −1.0 (300)
2[b]	−13.3 (233), +1.1 (272), −0.7 (310)
6	−12.3 (224), −12.8 (234), +0.8 (272), −1.4 (296)

a) See ref. 2. *b*) Spectra were measured in dioxane. *c*) See ref. 9. *d*) See ref. 4.

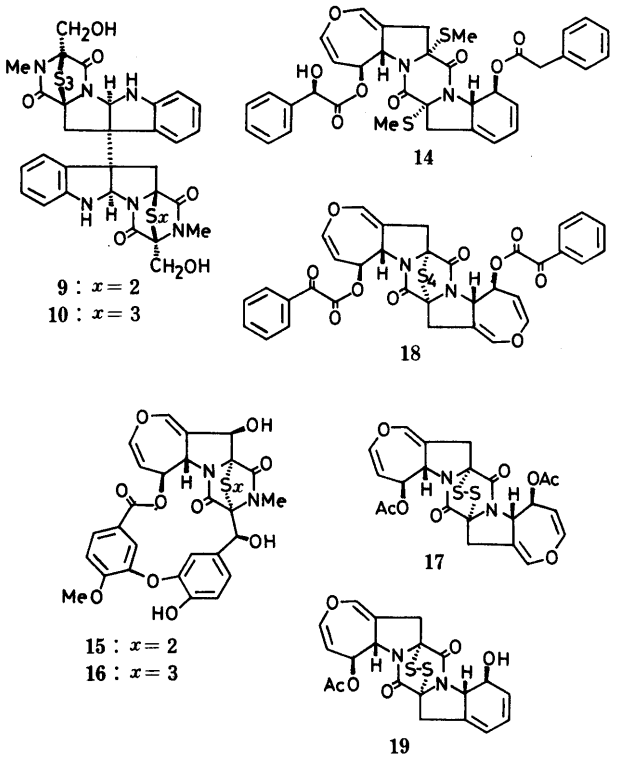

Chart 2

including the optical rotation, with the monoacetates of the naturally occurring emethallicins B (**1**) and A (**4**), respectively. Moreover, emethallicin B (**1**) was converted to deepitetrathiobis(methylthio)emethallicin B (**14**), $[\alpha]_D$ −140° ($CHCl_3$), $C_{36}H_{34}O_8N_2S_2$, by reductive methylation with $NaBH_4$ and CH_3I. This compound (**14**) was identical, including the optical rotation,[2] with deepidithiobis(methylthio)emethallicin A, derived from **4** by similar reductive methylation. Thus the absolute structures of emethallicin B (**1**) and emethallicin D monoacetate (**5**), and consequently emethallicin D (**3**) occurring naturally in *E. heterothallica*, were confirmed.

The circular dichroism (CD) spectra of the epipolythiodioxopiperazine derivatives, especially of epidithiodioxopiperazines, are expected to show maxima at 235, 270, 310, and 340 nm from a theoretical analysis.[8] The CD maxima of the disulfides, trisulfides, and tetrasulfides of emethallicins and emestrins are summarized in Table IV. The CD spectra of epidithiodioxopiperazines **4** and **7**,[2] and emestrin (**15**)[9] corresponded well to the above theoretical values. The positive Cotton effect observed at 301 and 293 nm for **15** and emestrin B (**16**), respectively, were due to the benzoate moiety.[4,9] The signs of CD maxima of epitetrathiodioxopiperazines **1** and **6** were still consistent with the theoretical ones, but the positions and the amplitudes of CD maxima were a little different from the theoretical ones. The trisulfide emethallicin D monoacetate (**5**) showed the maxima at 220 (negative), 244 (negative), and 298 nm (negative). The positive peak at 260—270 nm disappeared for **5**, and weak negative CD maxima at 310 and 340 nm for the disulfides were shifted to around 300 nm for the trisulfide **5** just as for the tetrasulfides **1** and **6**.

The ^{1}H-NMR and ^{13}C-NMR spectra of emethallicin C (**2**) (Tables II and III) showed almost half of the signals observed for emethallicins A (**4**)[2] and B (**1**), but the molecular formula of **2** was closely similar in size to those of **1** and **4**. These facts suggested that **2** has a symmetrical molecular structure. The ^{1}H-NMR signals of the basic

skeleton of **2** were similar, including their coupling patterns, to those of acetylaranotin (**17**) (Table III), isolated from *Arachniotus aureus* (EIDAM) SCHROETER,[10] as an antiviral antibiotic,[11] and *Aspergillus terreus* THOM.[12] The homonuclear ^1H–^1H shift correlation (^1H–^1H COSY) spectrum and the heteronuclear ^1H–^{13}C shift correlation (^1H–^{13}C COSY) spectrum of **2** also confirmed the basic skeleton of emethallicin C (**2**). Thus, it seems to be clear that emethallicin B (**2**) has the same basic skeleton as acetylaranotin (**17**), except for the presence of the tetrasulfide bond in **2** instead of the disulfide bond in **17**.

The ^1H-NMR and ^{13}C-NMR spectra of **2** (Tables II and III) suggested the presence of two mandelate moieties in the molecule of **2**. On oxidation with manganese dioxide, **2** afforded dioxoemethallicin C (**18**), $[\alpha]_D$ −345° ($CHCl_3$), $C_{34}H_{24}N_2O_{10}S_4$. The proton signals at δ 5.362, assigned to the protons attached to the carbon bearing the secondary hydroxyl groups in mandelates, and at δ 4.873, assigned as the hydroxyl group, in **2** disappeared in **18**. The above corresponding carbon signals at δ 72.51 for **2** showed an extreme downfield shift to δ 185.24 for **18**. From the above results, **2** and **18** were assumed to have two mandelic acid moieties, which would be connected at C-5 and C-13 of the basic skeleton.

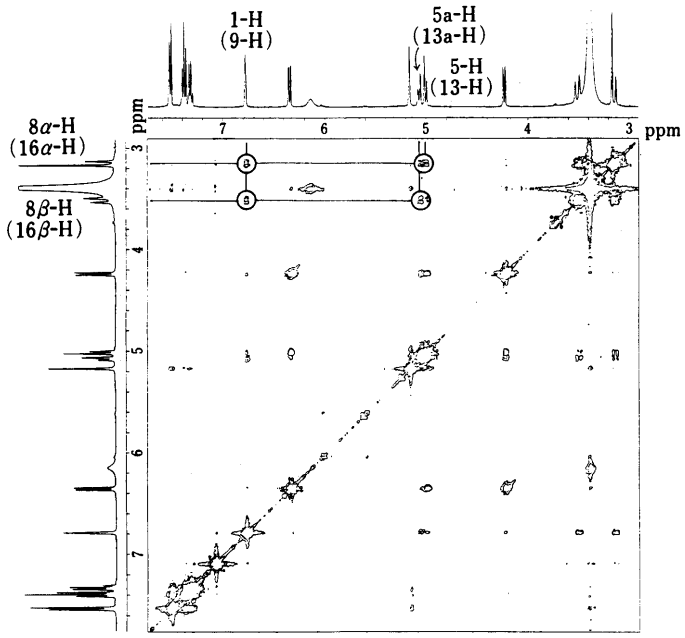

Fig. 1. ^1H–^1H NOESY Spectrum of Emethallicin C (**2**) in $(CD_3)_2SO$

In order to determine the stereochemistry of the basic skeleton of emethallicin C (**2**), the homonuclear ^1H–^1H nuclear Overhauser effect correlation (^1H–^1H NOESY) spectrum and the heteronuclear ^1H–^{13}C long-range shift correlation (^1H–^{13}C COLOC) spectrum of **2** were inspected. In the ^1H–^1H NOESY spectrum (Fig. 1), the correlation peaks of the upfield methylene proton signal at C-8 and C-16 (δ 3.144) were observed between the signals at δ 6.782 (1-H and 9-H) and 5.009 (5-H and 13-H), whereas the downfield methylene proton signal (δ 3.518) was well correlated with the signals at δ 6.782 (1-H and 9-H) and 5.079 (5a-H and 13a-H). Furthermore, the correlation peak was observed between the amide carbonyl carbon signal at δ 165.43 (C-7 and C-15) and the downfield methylene proton signal at δ 3.518 in the ^1H–^{13}C COLOC spectrum (Fig. 2), but no cross peak was observed between the above amide carbonyl carbon signal and the upfield methylene proton signal at δ 3.144. In the same spectrum, the carbon signal at δ 60.78 (C-5a and C-13a) was correlated only to the upfield methylene proton at C-8 and C-16. Therefore the ^1H-NMR signals at δ 3.518 and 3.144 were assigned to 8β-H (16β-H) and 8α-H (16α-H), respectively, and, consequently the relative stereochemistry of the basic skeleton of emethallicin C (**2**) was confirmed as being the same as that of acetylaranotin (**17**).

From the similarity of the CD curve of emethallicin C (**2**) [223 (negative), 272 (positive), and 310 nm (negative)] to that of emethallicin B (**1**) (Table IV), it is clear that the absolute stereochemistry of the epitetrathiodioxopiperazine moiety of **2** is the same as in emethallicins A (**4**), B (**1**), and D (**3**). In order to determine the absolute stereochemistry of the mandelates in the molecule of emethallicin C (**2**), **2** was hydrolyzed with aqueous NaOH followed by methylation with CH_2N_2 to give (−)-methyl mandelate, $[\alpha]_D$ −119° (MeOH),[13] whose absolute configuration is *R*.[14] The above results confirm that emethallicin C (**2**) should be, in its basic skeleton, the ester of (*R*)-mandelic acid in which the acid is linked dually at C-5 and C-13 to the deacetyl derivative of acetylaranotin (**17**).

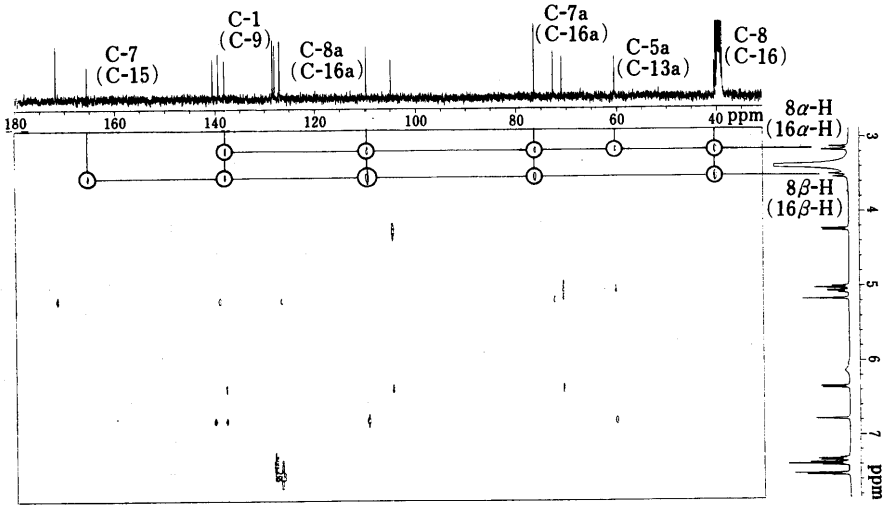

Fig. 2. ^1H–^{13}C COLOC Spectrum of Emethallicin C (**2**) in $(CD_3)_2SO$

Emethallicins B (**1**) and D (**3**), and C (**2**) have the same basic skeleton, including the absolute stereochemistry, as apoaranotin (**19**) and acetylaranotin (**17**), respectively, originally isolated from *Arachniotus aureus*[10,15] and *Aspergillus terreus*.[12] Although phenylacetic acid and its ester have been isolated from several fungi, emethallicins A—D (**1**—**4**) are the first examples of the isolation of mandelic acid, as a free acid and/or its ester, from fungi. The naturally occurring epitrithiodioxopiperazine and epitetrathiodioxopiperazine derivatives were usually isolated from fungi along with the corresponding epidithiodioxopiperazines as the main metabolite. In *E. heterothallica*, strain ATCC 16824 (mating type a), the main metabolic tetrasulfides [emethallicins B (**1**) and C (**2**)] were obtained along with the corresponding disulfide [emethallicin A (**4**)] and trisulfide [emethallicin D (**3**)], each isolated as the acetates (**7** and **5**) because of their small amounts in the extract. On the other hand, *E. heterothallica*, strain ATCC 16847 (mating type A), produced a large amount of the disulfide, emethallicin A (**4**).[2] Emethallicins B (**1**), C (**2**), and D (**3**) could not be isolated, though a small amount of **1** was detected on thin layer chromatography (TLC).

Emethallicins have potent inhibitory activities upon compound 48/80-induced histamine release from mast cells, and are also 5-lipoxygenase inhibitors, like emethallicin A (**4**).[2] The IC_{50} values for inhibition of histamine release were determined as 3.0×10^{-8},[2] 8.0×10^{-8}, 1.0×10^{-6}, 1.0×10^{-6}, 6.0×10^{-6}, and 2.0×10^{-8} M for emethallicins A (**4**), B (**1**), and C (**2**), emethallicin A monoacetate (**7**), emethallicin B diacetate (**6**), and emethallicin D monoacetate (**5**), respectively, whereas those for inhibition of 5-lipoxygenase were determined as 1.7×10^{-6},[2] 1.3×10^{-6}, and 2.6×10^{-6} M for **4**, **1**, and **2**, respectively. The inhibitory activity, toward histamine release, of the acetate is usually weaker than that of the original emethallicin, except for emethallicin D monoacetate (**5**) whose activity is as strong as that of **4**, although naturally occurring emethallicin D (**3**) could not be obtained at this time.[16]

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. Field desorption (FD) and FAB mass spectra (MS), using *m*-nitrobenzyl alcohol as the matrix, were taken with a JEOL JMX-HS-110 spectrometer. Ultraviolet (UV) and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JEOL IR-810 spectrophotometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JNM-GX-400 spectrometer at 399.78 MHz and at 100.43 MHz, respectively, or ^{1}H-NMR spectra were taken with a JEOL JNM-GX-270 spectrometer at 270.17 MHz, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet = s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m, and broad = br. Capital letters refer to the pattern resulting from directly bonded coupling ($^1H_{C,H}$). CD curves were determined on a JASCO J-600 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (LPLC) was performed on a Chemco Low-Prep 81-M-2 pump and glass column (150 or 200 × 10 mm) packed with Silica gel CQ-3 (30—50 μm; Wako). TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected by their absorption under UV light, and/or by spraying aqueous silver nitrate solution and then heating.[9]

Isolation of Emethallicins B (1) and C (2), and of D (3) as Its Acetate (5) from *Emericella heterothallica* *E. heterothallica* (mating type a), strain ATCC 16824, was cultivated at 27 °C for 14 d in 120 Roux flasks containing 250 ml of Czapek-Dox medium supplemented with 0.1% yeast extract in each flask. The dried mycelia (250 g) were pulverized and extracted with $CHCl_3$ at room temperature. The residue (16.0 g) obtained by evaporation of the extract was chromatographed on silica gel with $CHCl_3$ followed by rechromatography with benzene–acetone (30 : 1, v/v) to obtain the emethallicin A-rich fraction (300 mg) and with benzene–acetone (20 : 1, v/v) to give emethallicin B (**1**) (250 mg), and with $CHCl_3$–MeOH (50 : 1, v/v) to obtain emethallicin C (**2**) (50 mg). The above emethallicin A-rich fraction was acetylated at room temperature with acetic anhydride (2 ml) and pyridine (2 ml). The reaction mixture was poured into ice-water and extracted with AcOEt, and the solvent was evaporated. The residue was purified by LPLC using cyclohexane–AcOEt (5 : 1, v/v) to give emethallicin A monoacetate (**7**) (150 mg) and by repeated LPLC using cyclohexane–AcOET (4 : 1, v/v) to give emethallicin D monoacetate (**5**) (80 mg). Compound **7** was identical with the monoacetate derived from emethallicin A (**4**) isolated from *E. heterothallica* (mating type A), strain ATCC 16847.[2]

Emethallicin B (**1**): Colorless needles, mp 180—182 °C, from AcOEt–MeOH (1 : 1, v/v). $[\alpha]_D^{20}$ −268° (c = 0.30, $CHCl_3$). FD-MS *m/z* (%): 689 [$(M-S+1)^+$, 3], 593 [$(M-S_4+1)^+$, 49], 457 (59), 455 (53), 136 (100), 107 (20). FAB-MS *m/z*: 721 [$(M+1)^+$]. *Anal.* Calcd for $C_{34}H_{28}N_2O_8S_4\cdot 1/2H_2O$: C, 55.95; H, 4.01; N, 3.84; S, 17.57. Found: C, 56.00; H, 3.81; N, 3.70; S, 17.19. UV λ_{max}^{MeOH} nm (log ε): 259 sh (3.89), 265 sh (3.86), 284 sh (3.69). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1735, 1720 (COO), 1680 (CON). ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.075 (1H, d, J = 15.9 Hz, 8α-H), 3.101 (1H, d, J = 16.2 Hz, 15α-H), 3.451 (1H, br d, J = 15.9 Hz, 8β-H), 3.470 (1H, br d, J = 16.2 Hz, 15β-H), 3.713 (1H, d, J = 16.1 Hz, 2″-H), 3.764 (1H, d, J = 16.1 Hz, 2″-H), 4.241 (1H, dd, J = 8.2, 1.8 Hz, 4-H), 5.035 (1H, ddd, J = 8.4, 2.1, 1.8 Hz, 5-H), 5.083 (1H, br d, J = 8.4 Hz, 5a-H), 5.182 (1H, s, 2′-H), 5.261 (1H, br d, J = 13.7 Hz, 12a-H), 5.609 (1H, br d, J = 8.7 Hz, 10-H), 5.641 (1H, br d, J = 13.7 Hz, 12-H), 6.031 (2H, m, 9-H and 11-H), 6.337 (1H, dd, J = 8.2, 2.1 Hz, 3-H), 6.779 (1H, br s, 1-H), 7.24—7.37 (6H, m, aromatic protons), 7.395 (2H, br d, J = 7.0 Hz, 4″-H and 8″-H), 7.533 (2H, br d, J = 7.1 Hz, 4′-H and 8′-H). ^{1}H-NMR [$(CD_3)_2CO$] δ: 3.111 (1H, d, J = 15.7 Hz, 8α-H), 3.151 (1H, d, J = 16.1 Hz, 15α-H), 3.455 (1H, br d, J = 15.7 Hz, 8β-H), 3.496 (1H, br d, J = 16.1 Hz, 15β-H), 3.745 (1H, d, J = 15.7 Hz, 2″-H), 3.798 (1H, d, J = 15.7 Hz, 2″-H), 4.387 (1H, dd, J = 8.5, 1.2 Hz, 4-H), 5.221 (2H, br s, 5-H and 5a-H), 5.369 (1H, s, 2′-H), 5.397 (1H, br d, J = 13.3 Hz, 12a-H), 5.625 (1H, br d, J = 9.5 Hz, 10-H), 5.803 (1H, br d, J = 13.3 Hz, 12-H), 6.038 (1H, m, 11-H), 6.069 (1H, m, 9-H), 6.318 (1H, dd, J = 8.5, 2.4 Hz, 3-H), 6.661 (1H, br s, 1-H), 7.24—7.42 (8-H, m, aromatic protons), 7.670 (2H, br d, J = 7.2 Hz, 4′-H and 8′-H).

Emethallicin C (**2**): Pale yellow needles, mp 193—195 °C, from acetone. $[\alpha]_D^{20}$ −312° (c = 0.20, $CHCl_3$). FAB-MS *m/z*: 753 [$(M+1)^+$]. *Anal.* Calcd for $C_{34}H_{28}N_2O_{10}S_4\cdot H_2O$: 53.60; H, 3.84; N, 3.68; S, 16.84. Found: C, 53.67; H, 3.63; N, 3.64; S, 16.43. UV λ_{max}^{MeOH} nm (log ε): 230 sh (4.59), 290 sh (3.35). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1730 (OH), 1680 (CON). ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.144 (2H, d, J = 15.8 Hz, 8α-H and 16α-H), 3.518 (2H, ddd, J = 15.8, 1.8, 1.5 Hz, 8β-H and 16β-H), 4.237 (2H, dd, J = 8.2, 1.8 Hz, 4-H and 12-H), 5.009 (2H, ddd, J = 8.5, 1.8, 1.8 Hz, 5-H and 13-H), 5.079 (2H, ddd, J = 8.5, 2.1, 1.5 Hz, 5a-H and 13a-H), 5.167 (2H, br s, 2′-H and 2″-H), 6.348 (2H, dd, J = 8.2, 1.8 Hz, 3-H and 11-H), 6.782 (2H, dd, J = 2.1, 1.8 Hz, 1-H and 9-H), 7.30—7.40 (6H, m, aromatic protons), 7.529 (4H, br d, J = 6.7 Hz, 4′-H, 8′-H, 4″-H, and 8″-H). ^{1}H-NMR [$(CD_3)_2CO$] δ: 3.212 (2H, d, J = 16.4 Hz, 8α-H and 16α-H), 3.556 (2H, ddd, J = 16.4, 2.5, 1.7 Hz, 8β-H and 16β-H), 4.409 (2H, dd, J = 8.2, 2.0 Hz, 4-H and 12-H), 4.873 (2H, d, J = 5.6 Hz, 2′-OH and 2″-OH), 5.202 (2H, ddd, J = 8.5, 2.0, 2.0 Hz, 5-H and 13-H), 5.256 (2H, ddd, J = 8.5, 2.2, 1.7 Hz, 5a-H and 13a-H), 5.362 (2H, d, J = 5.6 Hz, 2′-H and 2″-H), 6.332 (2H, dd, J = 8.2, 2.0 Hz, 3-H and 11-H), 6.780 (2H, dd, J = 2.5, 2.2 Hz, 1-H and 9-H), 7.325 (2H, br t, J = 7.3 Hz, 6′-H and 6″-H), 7.391 (4H, br t, J = 7.3 Hz, 5′-H, 7′-H, 5″-H, and 7″-H), 7.667 (4H, br d, J = 7.3 Hz, 4′-H, 8′-H, 4″-H, and 8″-H).

Emethallicin D Monoacetate (**5**): White amorphous powder, $[\alpha]_D^{20}$ −269° (c = 1.14, $CHCl_3$). FAB-MS *m/z*: 731 [$(M+1)^+$]. *Anal.* Calcd for $C_{36}H_{30}N_2O_9S_3\cdot 1/3C_6H_{12}$: C, 60.14; H, 4.52; N, 3.69; S, 12.68. Found: C, 60.27; H, 4.59; N, 3.67; S, 12.61. UV λ_{max}^{MeOH} nm (log ε): 255 (3.90). IR ν_{max}^{KBr} cm^{-1}: 1740 (AcO, COO), 1700 (CON). ^{1}H-NMR ($CDCl_3$) δ (about 2 : 1 mixture of two conformers): 2.224 (3H, s, 2′-OAc), 2.893 and 2.880 (1H, br d, J = 17.3 Hz, 8α-H or 15α-H), 2.907 and 2.982 (1H, br d, J = 16.6 Hz, 15α-H or 8α-H), 3.414 and 3.387 (1H, br d, J = 17.3 Hz, 8β-H or 15β-H), 3.591 and 3.598 (1H, br d, J = 16.6 Hz, 15β-H or 8β-H), 3.799 and 3.745 (2H, br s, 2″-H_2), 4.314 and 4.266 (1H, dd, J = 8.3, 2.0Hz, 4-H), 5.014 and 5.129 (1H, br d, J = 8.1 Hz, 5a-H), 5.349 (1H, br d, J = 12.6 Hz, 12a-H), 5.523 (1H, br d, J = 7.5 Hz, 10-H), 5.769 and 5.323 (1H, ddd, J = 8.1, 2.0, 1.8 Hz, 5-H), 5.933 (2H, m, 9-H and 11-H), 6.170 and 5.982 (1H, s, 2′-H), 6.178 and 6.160 (1H, dd, J = 8.3 and 1.8 Hz, 3-H), 6.224 and 6.178 (1H,

br s, 12-H), 6.520 (1H, br s, 1-H), 7.22—7.43 (8H, m, aromatic protons), 7.567 and 7.586 (2H, br d, J=8.0 Hz, 4′-H and 8′-H); ^{1}H-NMR ($C_6D_5CD_3$ at 70 °C) δ: 1.896 (3H, s, 2′-OAc), 2.132 (1H, d, J=17.5 Hz), 2.387 (1H, d, J=17.5 Hz), 2.636 (1H, d, J=17.5 Hz), 3.208 (1H, br d, J=17.5 Hz), 3.784 (2H, br s), 4.306 (1H, br d, J=8 Hz), 4.877 (1H, br), 5.171 (2H, br), 5.338 (1H, br), 5.391 (1H, br d, J=9 Hz), 5.475 (1H, m), 5.759 (2H, m), 5.827 (1H, m), 6.228 (1H, br d, J=13 Hz), 6.434 (1H, br s), 6.97—7.21 (6H, m), 7.352 (2H, br d, J=8 Hz), 7.692 (1H, br d, J=8 Hz).

Acetylation of Emethallicin B (1) Emethallicin B (**1**) (100 mg) was dissolved in a mixture of acetic anhydride (1.5 ml) and pyridine (1.5 ml), and the solution was kept overnight at room temperature. The reaction mixture was poured into ice-water and extracted with AcOEt. The residue obtained by evaporation of the extract was purified by LPLC using benzene to give a monoacetate (**6**) (70 mg) and the starting material (**1**) (5 mg).

Emethallicin B Monoacetate (**6**): White amorphous powder. $[\alpha]_D^{25}$ −362° (c=0.98, $CHCl_3$). FD-MS m/z (%): 763 [$(M+1)^+$, 9], 634 [$(M-S_4)^+$, 20], 306 (100). *Anal.* Calcd for $C_{36}H_{30}N_2O_9S_4 \cdot C_6H_{12}$: C, 59.55; H, 5.00; N, 3.31; S, 15.14. Found: C, 59.36; H, 4.85; N, 3.34; S, 15.40. UV λ_{max}^{MeOH} nm (log ε): 264 (4.13). IR ν_{max}^{KBr} cm^{-1}: 1745 (OAc), 1730 (COO), 1695 (CON). ^{1}H-NMR ($CDCl_3$) δ: 2.251 (3H, s, 2′-OAc), 3.040 (1H, d, J=16.3 Hz, 8α-H), 3.067 (1H, br d, J=16.1 Hz, 15α-H), 3.243 (1H, br d, J=16.3 Hz, 8β-H), 3.289 (1H, br d, J=16.1 Hz, 15β-H), 3.750 (1H, d, J=15.6 Hz, 2″-H), 3.800 (1H, d, J=15.6 Hz, 2″-H), 4.365 (1H, dd, J=8.5, 1.2 Hz, 4-H), 5.227 (2H, br s, 5-H and 5a-H), 5.442 (1H, br d, J=15.4 Hz, 12a-H), 5.609 (1H, br d, J=9.0 Hz, 11-H), 5.819 (1H, br d, J=15.4 Hz, 12-H), 5.929 (1H, m, 10-H), 5.954 (1H, br s, 9-H), 6.131 (1H, s, 2′-H), 6.153 (1H, dd, J=8.5, 1.7 Hz, 3-H), 6.531 (1H, br s, 1-H), 7.254 (1H, br t, J=7.1 Hz, aromatic proton), 7.30—7.44 (7H, m, aromatic protons), 7.624 (2H, br d, J=6.8 Hz, 4′-H and 8′-H).

Reductive Methylation of Emethallicin B (1) $NaBH_4$ (20 mg) was added to a stirred solution of emethallicin B (**1**) (50 mg) in a mixture of MeOH (1 ml) and CH_3I (3 ml). After stirring for 1 h at room temperature, the reaction mixture was evaporated and extracted with AcOEt. The residue obtained by evaporation of the extract was purified by LPLC using $CHCl_3$ as solvent to give deepitetrathiobis(methylthio)emethallicin B (**14**) (20 mg) as colorless needles, mp 182—184 °C, from benzene. This compound (**14**) was identical with deepidithiobis(methylthio)emethallicin A[2] on the basis of a comparison of the IR and ^{1}H-NMR spectra, the optical rotations, and TLC behavior, and by the mixed mp.

Conversion of Emethallicin D Monoacetate (2) to Emethallicin A Monoacetate (7) and Emethallicin B Monoacetate (6) K_2CO_3 (200 mg) was added to a solution of emethallicin D monoacetate (**2**) (50 mg) in a mixture of acetone (2 ml) and MeOH (1 ml), and the mixture was stirred at room temperature for 30 min. After evaporation of the solvent, the residue was purified by LPLC with cyclohexane–AcOEt (5:1, v/v) to give emethallicin A monoacetate (**7**) (15 mg), with cyclohexane–AcOEt (4:1, v/v) to obtain recovered emethallicin D monoacetate (**5**) (10 mg), and then with cyclohexane–AcOEt (3:1, v/v) to give emethallicin B monoacetate (**6**) (20 mg). Compounds **7** and **6** here obtained were identical with authentic samples derived from emethallicins A (**4**) and B (**1**), respectively, by comparison of the spectral data and the optical rotations.[2]

Oxidation of Emethallicin C (2) with Manganese Dioxide MnO_2 (200 mg) was added to a stirred solution of emethallicin B (**2**) (50 mg) in $CHCl_3$ (3 ml). After 30 min, excess MnO_2 was filtered off and the solvent was evaporated. The residue was purified by LPLC with cyclohexane–$CHCl_3$ (1:1, v/v) to obtain dioxoemethallicin C (**18**) (40 mg).

Dioxoemethallicin C (**18**): Colorless needles, mp 198—200 °C, from benzene. $[\alpha]_D^{20}$ −345° (c=0.88, $CHCl_3$). FAB-MS m/z: 749 [$(M+1)^+$]. *Anal.* Calcd for $C_{34}H_{24}N_2O_{10}S_4$: C, 54.53; H, 3.23; N, 3.74; S, 17.13. Found: C, 54.26; H, 3.15; N, 3.72; S, 17.48. UV λ_{max}^{MeOH} nm (log ε): 255 sh (4.17). IR ν_{max}^{KBr} cm^{-1}: 1730 (COO), 1690, 1680 (CO, CON). ^{1}H-NMR ($CDCl_3$) δ: 2.995 (2H, d, J=16.5 Hz), 3.309 (2H, br d, J=16.5 Hz), 4.810 (2H, dd, J=8.3, 1.5 Hz), 5.407 (2H, m), 5.459 (2H, m), 6.358 (2H, dd, J=8.3, 2.1 Hz), 6.574 (2H, br s), 7.528 (4H, br t, J=7.3 Hz), 7.653 (2H, br t, J=7.3 Hz), 8.124 (4H, br d, J=7.3 Hz).

Hydrolysis of Emethallicin C (2) Followed by Methylation A 1 N NaOH solution (2 ml) was added to a stirred solution of emethallicin C (**2**) (30 mg) in acetone (2 ml). After 30 min, the reaction mixture was poured into water and extracted with AcOEt. The evaporated residue was methylated with CH_2N_2 in ether at room temperature. After removal of the ether, the residue was purified by LPLC with the solvent system of cyclohexane–$CHCl_3$ (1:1, v/v) to give (*R*)-methyl mandelate (4 mg), $[\alpha]_D^{20}$ −119° (c=0.20, MeOH). The above compound was identical with authentic (*R*)-methyl mandelate, $[\alpha]_D^{20}$ −143° (MeOH).[13,14]

Acknowledgements The authors are grateful to Dr. T. Sato and Dr. M. Chin of the Research Institute for Biology & Chemistry, Tsumura & Company, for testing the biological activities. We also thank Dr. Y. Ebizuka of the Faculty of Pharmaceutical Sciences, and Dr. Y. Ohashi of the Faculty of Medicine, University of Tokyo, and Dr. M. Kubo of the Research Institute for Biology & Chemistry, Tsumura & Company, for FAB and FD mass measurements. We are grateful to Mrs. T. Ogata and Mrs. M. Yuyama of the Faculty of Pharmaceutical Sciences, Hoshi University for elemental analyses and NMR measurements, respectively.

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